ANNUAL REVIEW OF PLANT PHYSIOLOGY

LEONARD MACHLIS, *Editor*
University of California

WINSLOW R. BRIGGS, *Associate Editor*
Stanford University

VOLUME 11

1960

PUBLISHED BY

ANNUAL REVIEWS, INC.

PALO ALTO, CALIFORNIA, U.S.A.

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA, U.S.A.

© 1960 by Annual Reviews, Inc.
All Rights Reserved

Library of Congress Catalog Card Number: A51-1660

FOREIGN AGENCY

Maruzen Company, Limited
6, Tori-Michome Nihonbashi
Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA
BY THE GEORGE BANTA COMPANY, INC.

PREFACE

Among the matters of general policy discussed at this year's meeting of the Editorial Committee was the recurrent criticism that in the *Annual Review of Plant Physiology,* unlike the parent *Annual Review of Biochemistry,* there is no "calendar regularity in reviewing a given subject." The reasons for this "opportunistic" selection of topics and titles were stated several years ago by the first editor, D. I. Arnon.[1] However, the selection of topics and titles has never been quite as opportunistic as might appear. The Editorial Committee has always worked closely to an outline of the field of plant physiology and always considers whether for each main heading and subheading of the outline a review is appropriate.

We decided that beginning with this volume the main headings of this outline would be included in the Contents. We hope that this will facilitate the location of reviews in a particular subject area. Of course, there will inevitably be some reviews which cannot be neatly assigned a position since they will have been selected because of the topical appeal of a particularly active area of research rather than for their place in the overall plan.

Having taken the decision to publish the outline that had been followed for so many years, we believed it necessary to submit it to a careful reappraisal. The resulting revised outline appears in this volume in the Contents and in a new index (see below). The eleven headings in their assigned order appear complete in the index; some are missing from the Contents because either no review was planned or an expected review failed to materialize.

Another compromise with reality has resolved a second perennial problem—the desire for cumulative indexes. Cumulative indexes have been published for the *Annual Review of Biochemistry* but proved to be financially impracticable. The solution reached consists of two parts. Firstly, all previous reviews published as well as those in this volume are listed at the end of the book under the main outline headings referred to above (Cumulative Index of Chapter Titles). A number of the reviews are entered under more than one heading. Future volumes will continue listing past reviews going back at least five years and perhaps more. Secondly, the authors of the reviews so included are separately indexed (Cumulative Index of Contributing Authors). We hope these two new indexes will facilitate the use of the *Annual Review of Plant Physiology.*

One section of every preface, at least, is repetitive with calendar regularity—a statement of our debts for the successful appearance of another volume. First of all, we acknowledge with gratitude the meritorious service of John G. Torrey as Associate Editor for the past four years. We welcome as his replacement Winslow R. Briggs. We also bid farewell to Kenneth

[1] *The Annual Review and the Boundaries of Plant Physiology* by D. I. Arnon, Ann. Rev. of Plant Physiol. 6: v-viii, 1955.

Thimann at the end of his second distinguished term on the Editorial Committee and look forward to the contributions to be made by his successor, Folke Skoog.

As is true each year, the hard work resulting in publication rests heavily on our authors, on our editorial assistants, who for this volume were Anne Bogley and Lois Cox, our indexer, Grace MacNeill, and our publisher, the George Banta Company.

H. B.
W. R. B.
F. E. G.
S. B. H.
J. G. H.
A. L.
L. M.
F. S.

CONTENTS

PAGE

CELL STRUCTURE AND FUNCTION:
THE SUBMICROSCOPIC STRUCTURE OF THE CELL, *Frank Mercer* . 1
NITROGEN METABOLISM:
NITROGEN FIXATION, *Conrad S. Yocum* 25
PHOTOSYNTHESIS:
ENERGY TRANSFER THROUGH PHOSPHORYLATION MECHANISMS IN PHOTOSYNTHESIS, *Otto Kandler* 37
THE PRIMARY PROCESSES OF PHOTOSYNTHESIS IN PLANTS, *A. A. Krasnovsky* 363
GENERAL METABOLISM:
BIOSYNTHETIC PATHWAYS OF AROMATIC COMPOUNDS, *A. C. Neish* 55
CRASSULACEAN ACID METABOLISM, *S. L. Ranson and M. Thomas* 81
WATER RELATIONS:
HEAT TRANSFER BETWEEN THE PLANT AND THE ENVIRONMENT, *K. Raschke* 111
THE RELATIONSHIP BETWEEN SALT UPTAKE AND THE ABSORPTION OF WATER BY INTACT PLANTS, *R. Scott Russell and D. A. Barber* 127
ABSORPTION AND TRANSLOCATION:
TRANSPORT IN THE XYLEM, *E. G. Bollard* 141
TRANSPORT IN THE PHLOEM, *Martin H. Zimmermann* 167
GROWTH AND DEVELOPMENT:
VERNALIZATION AND ITS RELATIONS TO DORMANCY, *P. Chouard* . 191
THE MECHANISM OF ACTION OF AUXIN, *A. W. Galston and W. K. Purves* 239
GIBBERELLINS AS NATIVE PLANT GROWTH REGULATORS, *B. O. Phinney and Charles A. West* 411
PATHOLOGICAL PHYSIOLOGY:
SOME ASPECTS OF THE STRUCTURE AND BEHAVIOR OF TOBACCO MOSAIC VIRUS, *Albert Siegel and S. G. Wildman* 277
PECTIC AND CELLULOLYTIC ENZYMES IN PLANT DISEASE, *R. K. S. Wood* 299
SPECIAL TOPICS:
PHYSICS AND CHEMISTRY OF PROTOPLASMIC STREAMING, *N. Kamiya* 323
PRODUCTIVITY OF THE OCEANS, *E. Steemann Nielsen* 341
INDEXES:
AUTHOR INDEX 437
SUBJECT INDEX 452
CUMULATIVE INDEX OF CONTRIBUTING AUTHORS (VOLUMES 1 THROUGH 11) 461
CUMULATIVE INDEX OF CHAPTER TITLES (VOLUMES 1 THROUGH 11) 463

Annual Reviews, Inc., and the Editors of its publications assume no responsibility for the statements expressed by the contributors to this *Review*.

THE SUBMICROSCOPIC STRUCTURE OF THE CELL[1]

By Frank Mercer

Department of Botany, University of Sydney, Sydney, Australia

Recent developments in our knowledge of the submicroscopic structure and organization of the plant cell have resulted almost entirely through developments in electron microscopy, ultra microtomy, and cell fractionation techniques. Some idea of the tremendous activity and ever-growing interest in electron microscopy is illustrated by the fact that many hundreds of papers in this field are being published in 1959, and without question the number published in the future will be enormous.

Fortunately for the research worker the problem of keeping aware of significant developments and better techniques in the field is relatively easy because of the existence of N.Y.S.E.M. (56) and the publication of a number of journals almost entirely devoted to electron microscopy and cognate subjects (4, 36). N.Y.S.E.M. is a card index compiled by the New York Society of Electron Microscopists and published quarterly. Its purpose is to catalogue all literature on electron microscopy. Thus, since excellent literature sources are available to specialist and non-specialist alike, there seems little point in undertaking an exhaustive review of the literature. Rather, the review can serve the purpose of presenting a generalized picture of current views on certain aspects of cell structure which are relevant to plant physiology.

The currently popular approach to problems of structure and function at the cell level involves a combination of electron microscopy and cell fractionation techniques. Differential centrifugation provides a means of separating plant cells into various fractions: those described by the light microscopists; others, such as the microsomes and endoplasmic reticulum which are not seen under the light microscope; and possibly also a heterogeneous collection of artifact fractions arising from the homogenized cell contents.

In general, cells are disrupted in an isotonic medium at low temperature and the various fractions are removed by centrifugation at different speeds. After a few minutes at about 200 $\times$ g, cell walls are sedimented, at 1000 to 2000 $\times$ g, nuclei, and at 10,000 to 15,000 $\times$ g, mitochondria. Endoplasmic reticulum and microsomes are sedimented after about 30 min. at 100,000 $\times$ g. These fractions may be studied and characterized in biochemical, biophysical, or physiological terms. The standard fractionation procedures are well known and have been evolved through the activities of numerous investigators.

[1] The survey of literature pertaining to this review was concluded June 1959.

Electron microscopy provides a means of studying the structure of these cell fractions, as well as their structure *in situ*. Thus, comparisons can be made between isolated systems and intact tissues, and a conception of structure and function at the cell level developed. The standard electron microscope techniques have been described recently by Sjöstrand (75).

Unfortunately the preparation of material for electron microscopy is rather drastic, and no doubt gives rise to artifacts. Electron microscopy, like light microscopy, is thus plagued by some uncertainty. Tissue, or isolated cell-fractions are killed with fixatives such as osmium tetroxide, potassium permanganate, etc.; dehydrated through an alcohol series; embedded in a resin, and sectioned. The sections must be thin (a few hundred Angstrom units), since electrons can not pass through thick layers of matter, and the material must be examined in vacuum because electrons can not pass through air.

This preparative treatment combined with the vacuum experience, introduces an element of uncertainty into electron microscope data. The probability exists that the killing process and subsequent treatment alter fine structure either by rendering parts of the cells soluble or by precipitating and coagulating cell contents. Obviously, the comparative crudity of existing techniques must lead to differences of interpretation. Whether a structure is artifact or not must always be considered. Despite the drawbacks, the combination of electron microscopy and cell fractionation is likely to prove one of the most powerful research tools in the history of botany. Already it has revealed an intricacy of structure unsuspected a few years ago, and basic problems of organization and function are being elucidated.

In the mid-thirties the cell organelles and cytoplasm, because of their optical homogeneity and other properties, were assumed to be devoid of structure. Both mitochondria and chloroplasts were pictured as homogeneous structure enclosed by a semipermeable membrane, and the cytoplasm was likened to an aggregation of protein molecules reversibly linked to give a loose reticulum (26, 27).

Electron microscopy has shown that these interpretations of cell structure are wrong. It is now realized that organelles and cyotplasm have an elegant and intricate submicroscopic structure beyond the resolution of the light microscope. This review will describe some of the more important findings concerning the organelles and cytoplasm with particular reference to their ontogeny and possible significance to cell physiology.

Membranes and the Endoplasmic Reticulum

Many physiological processes, such as cell permeability, accumulation, transport, diffusion of solutes, and osmosis, depend upon the nature of the barriers separating internal and external environments of the cell. Until recently the only sites accepted as diffusion barriers were the ectoplast (the external cell membrane) and the tonoplast (the vacuolar membrane). The

existence of the ectoplast and the tonoplast, both as cytological and physiological units, has been accepted by botanists for decades. Evidence for these structures is found in many textbooks and need not be discussed in this review. Models of their molecular architecture have been described and they are pictured as having a basic structure of lipid and protein films (19).

On the basis of its optical homogeneity, non-Newtonian properties, and sol-gel transformations, the cytoplasm has been assumed to have a molecular structure of linear protein units capable of cross linking to give a more or less rigid reticulum or a more fluid matrix, or both (26, 27). The significant point about this concept is that cytoplasm without structure (except at the molecular level) is enclosed between two structurally distinct filmlike membranes, the ectoplast and tonoplast, which control the movement of substances within the cell and between the cell and the external environment.

In recent years physiological evidence has accumulated which suggests that part of the cytoplasm is freely available to externally applied electrolytes or non-electrolytes. The volume of tissue into which an external solute can penetrate by diffusion can not be explained unless a large part of the cytoplasm is freely available to the solute [Briggs & Robertson (11)]. The volume of freely available tissue is termed the apparent free space, and the volume not available is termed the apparent osmotic volume. The significant conclusion from this work is that the external layers, as well as much of the cytoplasm, do not function as barriers to diffusion, but that a barrier does exist between the regions of apparent free space and apparent osmotic volume. That is, the ectoplast has no existence as a physiological membrane, whereas a physiological membrane does separate regions of the apparent osmotic value and the apparent free space of the cell. The observations on apparent osmotic value demonstrate that the membrane between apparent free space and apparent osmotic value has semipermeable properties in a sugar-water medium, and differentially permeable properties in a sugar-alcohols-water medium [Mercer (50)]. According to this concept of the protoplast, the structureless cytoplasm (excepting at a molecular level) is pictured as freely exposed on its external surface but separated from an osmotic phase by a physiological membrane. The membrane could correspond to the tonoplast and the osmotic phase with the vacuole.

Studies with the electron microscope have yielded information which may help to reconcile these divergent views and be of considerable importance to the understanding of all physiological processes which involve the movement of solutes.

Practically all electron micrographs published to date of sections of fixed cells show the boundary between vacuole and cytoplasm as a dense, frequently double line about 70 to 100 A in thickness. The boundary resembles a thin ribbon in certain orientations and gives the impression of forming a continuous film around the vacuole. This structure corresponds in position to the tonoplast. A double membrane, about 70 to 100 A in

thickness, has also been described at the external surface of the protoplast in contact with the cell wall in a number of different cells. This structure corresponds in position to the ectoplast. The existence of these membranes has been mentioned in many electron microscope studies of plant cells (12, 34, 46 to 48, 69, 70, 73).

In turgid tissue the ectoplast is not readily identified and its dimensions are difficult to determine, but it is clearly seen in plasmolysed tissue or in cells in which the protoplasm has retracted from the cell wall [McLean (47); Mercer (48)]. This difference might be because of the difficulty of resolving the boundary between wall and membrane in turgid tissue. Or, one might argue that the membrane forms as a precipitation membrane when the surface of the protoplast becomes exposed during plasmolysis or retraction. The latter explanation is not likely to apply to the structure identified as the tonoplast. If the tonoplast as seen in electron micrographs is accepted as representing a real structure, then on the same grounds the ectoplast should be accepted as a real structure.

As far as has been determined at the present moment, the two membranes appear to have an identical structure under the electron microscope. This statement is particularly true for plasmolysed cells (47, 48). Both membranes are between 70 to 100 A in thickness, frequently double, apparently homogeneous, and they have the same relative electron density. The electron microscope observations, therefore, suggest the existence of cytological membranes, the ectoplast and tonoplast, enclosing the protoplasm. Insufficient observations have been made to decide whether or not such membranes form unbroken continuous films, as expected according to the classical picture of the protoplast; and some observations suggest that both membranes may not exist as continuous films. This latter possibility will be considered after a description of recent observations on the structure of the cytoplasm.

The classical picture of a structureless cytoplasm, or rather a cytoplasm with a molecular continuum of linear molecules, is no longer tenable. Electron microscopy has demonstrated in the cytological sense the existence of a structural framework within the cytoplasm. This framework is beyond the resolution of the light microscope, but of larger than molecular dimensions. As this picture was described first in animal cells, and because far more information exists about animal than plant cytoplasm, a generalized description of the current view on the structure of animal cytoplasm is not out of place here [Porter, Claude & Fulham (65); Porter (66); Palade & Porter (57); Palade (58 to 60; 62)].

Cytoplasm of animal cells apparently consists of two structural regions separated by a continuous membrane system called the endoplasmic reticulum. The reticulum, according to Palade (59), forms a continuous network of membrane-bound cavities permeating the entire cytoplasm from cell membrane to nucleus. The membranes of the nucleus and golgi bodies, and the cell membrane are believed to be continuous with the membranes of the

endoplasmic reticulum. A relatively homogeneous or granular matrix occurs within the spaces enclosed by the membranes of the reticulum, while the cytoplasmic regions outside the reticulum apparently contain little electron-dense material.

Small, approximately spherical granules, 100 to 200 A, are frequently but not invariably attached to the membranes of the reticulum. Within the same cell, occur regions of the reticulum that are free of granules, and others where the granules are concentrated. The granules are rich in RNA and are thought to be the microsomes (57 to 60; 65, 66).

Thus the cytoplasm of the animal cell is pictured as consisting of two structural phases separated by a continuous membrane system: one phase contains the cytoplasmic matrix and is continuous with the nucleus; and the other phase, apparently more or less empty, is continuous with the external environment. This description is a generalized account. Variations in extent and volume of the endoplasmic reticulum occur with cell type but do not alter the basic picture. As this picture of cytoplasm is revolutionary it is of considerable importance to see how much of it applies to plant cells.

Insufficient data exist to decide whether or not the basic structure of plant cytoplasm is identical with that observed in animal cells, but enough observations have been made to suggest that plant and animal cytoplasm is essentially similar.

Endoplasmic reticulum has been identified in many different plant cells (12, 34, 44, 47, 49, 70, 72, 73). As in animal cells, it consists of a three-dimensional system of membrane-bound cavities in a finely granular matrix. Microsomes occur attached to the membranes or randomly distributed through the granular matrix. The amount of reticulum in plant cytoplasm appears to be less than in animal cells. It varies in total volume, intracellular distribution, and structural details from one cell type to another, but a complete spectrum of endoplasmic reticulum types has not yet been described for plants.

In some meristematic cells the volume of reticulum is small, possibly nil, but the finely granular matrix contains numerous microsomes. The microsomes in these tissues are not, apparently, associated with the membranes of a reticulum (16, 46, 73). Possibly the reticulum differentiates later in the growth of the cell. Support for this suggestion is shown by the changes observed in cytoplasmic structure during the growth of the internode cells in *Nitella* and *Chara* (34, 47). The cytoplasm of the meristematic cell contains numerous spherical vesicles, 0.1 to 0.3μ in diameter, but no endoplasmic reticulum. Mature cells contain endoplasmic reticulum and few vesicles. Similar conclusions were reached by Sitte (73) for the development of the reticulum in *Pisum sativum*, and developmental changes have been shown by Lund, Vatter & Hanson (46) for *Zea* roots. Although only a few cell species have been examined, the observations justify the conclusion that the endoplasmic reticulum is a characteristic intracellular structure in the cytoplasm of plant cells.

Some electron microscope observations suggest that continuity between the membrane systems of the protoplast does occur. The nuclear membrane in plants is double and in this respect resembles the nuclear membrane of animal cells [Watson (82)]. Continuity between nuclear membrane and vesicular elements is reported in *Triticum* (47). Sager & Palade (70) and Buvat (12) are convinced that continuity exists between all membrane systems. In *Chlamydomonas* (70) two membrane systems, endoplasmic reticulum and dictyosomes, have been identified. Apparently the two systems form part of a continuous membrane system between the endoplasmic reticulum and limiting membrane on one side, and the nuclear and chloroplast membrane on the other side. Also, elements of the dictyosomes appear continuous with the membranes of the central vacuole. A similar picture is described by Buvat (12) for meristematic cells of *Elodea canadensis*. These cells contain endoplasmic reticulum and dictyosomes, and continuity appears to exist between these systems and the nuclear membranes and ectoplast.

Biochemical continuity between nucleus and cytoplasm is suggested by the presence of microsomes in nucleus and cytoplasm. Microsomes isolated from the nuclei of pea stems are identical with those from the cytoplasm of the same tissue in their sedimentation constants, RNA content, and base ratios of RNA (8, 81). It is not known whether biochemical continuity would necessarily involve the nuclear membrane and endoplasmic reticulum.

Membrane-bound vesicles in contact with the ectoplast and tonoplast are a common feature of plant cytoplasm. Whether these vesicles are isolated cytoplasmic vacuoles, whether they are continuous with the endoplasmic reticulum, or whether they are artifacts arising from the disorganization of the cytoplasm during fixation is not known. Vesicles can arise as artefacts through faulty fixation and osmotic disorganization [Hodge (36)]. Alternatively they may correspond with invaginations connecting endoplasmic reticulum and ectoplast to give a region of the cytoplasm separate from the cytoplasmic matrix, as in animal cells. Buvat's (12) electron micrographs of *Elodea* showing invaginations of the ectoplast in the cytoplasm appear convincing. How far this feature would apply to both the tonoplast membrane and the ectoplast membrane remains an open question.

Apart from the observations for *Chlamydomonas* (70) the chloroplast membranes and mitochondrion membranes appear to be true limiting membranes and not continuous with membranes in the cytoplasm. Evidence, though meagre, supports the possibility of structural continuity between the membranes of the endoplasmic reticulum, nuclear membrane, dictyosomes, ectoplast, and to a lesser extent the tonoplast.

Most electron micrographs in the literature give the impression that the tonoplast is independent of the endoplasmic reticulum. Cytoplasmic vesicles in contact with the tonoplast do occur, but despite this feature the tonoplast appears to form a continuous film enclosing the vacuolar sap. The tono-

plast apparently seals the contents of the vacuole from the cytoplasm and endoplasmic reticulum.

From other data one could argue that the membranes enclosing the cytoplasmic vacuoles, the endoplasmic reticulum, the ectoplast, and the tonoplast have the same basic molecular structure. Their dimensions cover the same range of thickness, 70 to 100 A, their appearance under the electron microscope is similar, they have the same relative electron density and are frequently double—two dense lines separated by an "empty space." Does this similarity imply continuity of a three-dimensional membrane system, or are all biological membranes simply built according to the same molecular plan?

Comparatively little direct evidence has been obtained about the physicochemical properties of the membranes, particularly the tonoplast and ectoplast. A cell fraction, isolated by centrifugation from the mitochondria-free supernatant previously obtained from various plant tissues, contains lipid, protein, and RNA. Electron microscopy observations show that such material consists of membrane-bound vescicles, membrane fragments, and microsomes both free and attached to membrane fragments (8, 35, 46, 59). The presence of the microsomes is good proof that much of the fraction isolated at high speeds is derived mainly from the endoplasmic reticulum. As the RNA content of endoplasmic membrane is probably attributable to contamination from microsomes, it is concluded that the basic components of the endoplasmic membranes are lipids and proteins. Since much of the membrane is insoluble in the aqueous suspension medium, at least some of the structural protein is likely to be of an insoluble, possible fibrous, type.

No analytical data on the empirical composition of the tonoplast and ectoplast exist. Conclusions about their structure from classical studies on permeability are of limited value since the possibility that the endoplasmic reticulum has well defined permeability properties is a source of confusion. However, one can accept the overall conclusion that lipoprotein membranes are involved in permeability even though their exact site is questioned.

Some direct information on the nature of the tonoplast has come from studies with isolated vacuoles [Chambers & Höfler (14); Clark (17); McLean (47)]. Vacuoles may be isolated by allowing protoplasts to expand. Frequently the cytoplasm ruptures and sloughs off, extruding the vacuole. As far as has been ascertained the vacuole consists of a tonoplast enclosing vacuolar sap, but possibility of absorbed protein or lipid has not been excluded. Such isolated vacuoles have semipermeable properties in a sugar-water medium, obey the osmotic relation $P(V - b) = K$ and have differentially permeable properties in sugar-water media containing fat-soluble solvents. The isoelectric point of the surface, measured by electrophoresis, is *circa* pH 2.9 in media of low ionic strength (0.01). The shape of the mobility-pH curve suggests that amphoteric molecules are responsible for the electrophoretic properties of the surface. As the isoelectric point of cyto-

plasmic proteins is usually in the range pH 4 to 6 (18, 27) and since phospholipids have an isoelectric point at pH 3 (27), the surface of the tonoplast may contain phospholipid. Such evidence is not inconsistent with a lipoprotein structure for the tonoplast. The apparent ease with which vacuoles become detached from the cytoplasmic phase may mean that the tonoplast is not structurally linked to the cytoplasm. That is, the tonoplast exists as a lipoprotein membrane separating the vacuolar phase from the cytoplasm. This conclusion is supported by Sitte's observation (73) that myelin figures, with a 33 A period, are formed from the tonoplast. Similar data for isolated protoplasts indicate that their surface contains phospholipid and, in conjunction with their electron dense nature, could be lipoprotein (47).

Existing data indicate that endoplasmic reticulum, ectoplast, and tonoplast are basically lipoprotein structures; but this is not proof that they are part of a common membrane system. Information on this possibility awaits the comparison of serial sections of a single cell.

Since many structural features of plant and animal cells are similar, the tentative conclusion reached by McLean (47) that the basic structure of plant cytoplasm is similar to animal cytoplasm seems justified, with the possible difference that the tonoplast may not form part of the continuous membrane system but exists as a barrier separating the vacuolar phase from the cytoplasmic phase. If this conclusion is valid, then plant cytoplasm can be visualized as two main regions separated by the endoplasmic reticulum: one phase is continuous with the nucleus and the other phase is continuous with the external environment. This concept of the protoplast has important consequences for many problems of cell physiology and would go part way towards reconciling the cytological and physiological concepts of the cell described at the beginning of this review. A certain volume of the cytoplasm, namely, that volume outside the anastomoses of the endoplasmic reticulum and ectoplast, could behave as free space. An externally applied solute could diffuse into this volume, but without crossing the continuous ectoplast-endoplasmic reticulum cell membrane which could still retain its classical properties. The cell therefore would have apparent free space properties as well as classical permeability properties.

Similarly, the existence of a structural free space possibly filled with liquids and solutes could have a bearing on the interpretation of the electrical properties of cells and tissue. Electrodes inserted into cytoplasm would disturb the membrane system and it might be difficult to correlate the measurements with a particular phase of the cell.

The concept presented above may be significant to problems of solute accumulation and transport. Various investigators have speculated concerning the physiological role of the endoplasmic reticulum in animal cells [Bennett (5, 6); Palade (59)]. It is suggested that transport of a solute may occur via a process pictured as the function of a property of the reticulum. Invaginations of the membranes are followed by a budding of vesicles which subsequently break down to release their contents. The suggested mechanism is pinocytosis at the submicroscopic level. In this way

substance could be transported without penetrating the membranes. Is this the significance of the vesicles which occur in contact with ectoplast and tonoplast in plant cells?

Microsomes

Another important development in the submicroscopic field has been the morphological identification of the microsome, the establishment of its role in protein synthesis, and the possibility that microsomes carry the genetic message from nucleus to cytoplasm.

Only a few years ago, microsomes (and lyosomes) were believed to be a biochemically heterogeneous system (21, 41, 51, 60). The observations on which this belief was based were made on cell fractions isolated from mitochondria-free supernatants obtained by high speed centifugation. Microsomes, therefore, were defined as a fraction sedimenting in a particular centrifugal field. Electron microscopy has made possible the comparison of the structure of these fractions with the structure of the intact cell.

Microsome preparations have been shown to consist of membrane-bound vesicles, membrane fragments, and small spherical particles 100 to 300 A in diameter both free and attached to membranes [Palade & Siekevitz (61); Hodge, Martin & Morton (35); Huff, Hogeboom & Dalton (41)].

The fact that the endoplasmic reticulum consists of membranes frequently associated with small spherical particles indicates that the microsome fraction is possibly derived from the endoplasmic reticulum. Obviously the kind of microsome isolated by centrifugation will depend both on the extent to which the endoplasmic reticulum is fragmented by the preparative treatment, and on whether different types of endoplasmic reticulum exist. This would account for the well-known biochemical heterogeneity of the microsomal fractions.

Two or three years ago the microsome fraction was considered to consist of two structurally different components, membranes and dense spherical particles [Palade (60)]. Improved fractionation techniques, combined with the fact that the membranes of endoplasmic reticulum are not well developed in some plant cells, has led to the isolation of pure preparations of the dense spherical particles. Preparations have been obtained from bacteria, yeast, and animal tissues and from cytoplasm and nuclei of a single species (pea). The particles have been characterized in terms of sedimentation constants, shape and diameter, and RNA content. They appear to be essentially similar in the different organisms and are described as microsomes (8, 15, 45, 71, 81).

Tso [(81) and cited by Bonner (8)] has shown that the microsome is composed of sub-units. After removal of magnesium, microsomes from pea stem and root disaggragate into six sub-units each consisting of RNA and structural protein, and the sub-unit is claimed to be equivalent to a single giant RNA molecule.

The association between RNA and protein synthesis has been known for many years [Caspersson & Schultz (13)]. Later the microsome fraction was

recognized as capable of protein synthesis (9, 51, 60), and it was established that RNA is synthesized in the nucleus (2, 9, 44). Finally, since microsomes (RNA bodies) have been observed in, and isolated from, nucleus and cytoplasm, Bonner suggests that microsomes are synthesized in the nucleus and migrate into the cytoplasm, presumably through the pores of the double nuclear membrane (8). (As mentioned previously this may be evidence in favour of the continuity of the nuclear membrane and the endoplasmic reticulum.) It is possible that after 50 years chromidia may become a reality in the form of the microsome.

Assuming that protein is formed on the RNA surface of the microsomes it is probable that one, or at the most two species of protein can be formed at that surface. If this assumption holds, there must exist within the cell many different species of microsomes which are superficially alike (8). That is, the microsomes form a functionally homogeneous group at the general level of activity—protein synthesis, but are a heterogeneous group at the specific level—the synthesis of particular proteins. At the present level of resolution electron microscopy would not resolve such heterogeneity in terms of morphological types.

Mitochondria

It is now firmly established that mitochondria from many different cell types, plant, animal, and microorganisms have a common biochemical and common structural denominator. Mitochondria can be identified either *in situ* or in cell fractions purely on gross structural grounds. This is a significant advance for it opens the way for an attack on the correlation of biochemical properties with structure, and of assessing the purity of cell fractions isolated by differential centrifugation.

On the biochemical side, where information about the properties of mitochondria is impressive, the mitochondria can be defined accurately in biochemical terms as bodies capable of bringing about the reactions of the Krebs cycle, fatty acid oxidation, and oxidative phosphorylation (28, 30, 31, 51, 52).

Whether or not different kinds of mitochondria exist has not been resolved. Biochemical data have demonstrated that differences exist between various mithochondrial preparations. Whether these differences reflect a real biochemical heterogeneity amongst the cells' populations of mitochondria or whether they are artifacts inherent in cell fractionation techniques is not known. Eventually, improved isolation procedures combined with electron microscopy may give unambiguous data [Novikoff (55)].

A few observations are consistent with the existence of several kinds of mitochondria. Heterogeneity is suggested in particular by the differences in oxidative activity of mitochondrial preparations from young and mature fruit of *Capsicum*, and from young and mature pine seedlings [Howard & Yamaguchi (38); Stanley & Conn (76)].

The existence of highly integrated enzyme systems within the mitochondrion implies the existence of a high level of organization. Until recently the nature of this organization seemed likely to be found from bichemical properties, such as concentrations of reactants, kinetics of enzyme reactions, composition of the internal environment of the mitochondrion, and so on. There seemed little possibility of other kinds of organization, such as structural and spatial distribution of the reactants and the various enzymes, since the mitochondrion was assumed to be a structureless sac. [Frey-Wyssling (*27*)]. Yet the complexity of function implies a complexity of structure. This apparent anomaly, the lack of structural organization in an integrated enzyme system, has disappeared. Electron microscope studies have shown that the mitochondrion has an elegant and characteristic structure that is beyond the resolution of the light microscope.

Despite differences in size and shape of mitochondria, whether rod-shaped, filamentous, or spherical, and differences in phylogenetic position, etc., the basic structure is similar in a wide range of plants; yeast, green algae, ferns, and higher plants (1, 4, 24, 46 to 48, 63, 69, 70, 73, 84). The only exception is seen in the blue-green algae where mitochondria have not been identified (10, 47). Consequently a generalized description of the basic structure as seen in section *in situ* can be given.

The mitochondrion in fixed tissue is enclosed by the mitochondrial membrane which consists of two, usually parallel electron-dense layers, each *circa* 40 to 60 A in thickness, and separated by a less electron-dense space *circa* 60 to 90 A wide. The outer of the two layers surrounds the mitochondrion as a limiting membrane. The inner layer is continuous with an internal membrane system, composed of the cristae mitochondriales which project into the body of the mitochondrion. The cristae may be infolds of the inner layer and their distribution, number, length, and shape vary in different sections and in different mitochondria. In plant mitochondria the cristae are less well developed than in animal mitochondria and tend to have a more random orientation. In section, the cristae often give the impression of being vesicular or tubular in shape rather than plate or disclike structures (32). The shape of the cristae may vary according to the ontogenetic stage, phylogenetic status, physiological activity, and other factors.

When sectioned, cristae show a structure similar to the mitochondrial membrane. They consist of two parallel dense layers, or membranes, *circa* 40 to 60 A in thickness and are continuous with the inner layer of the mitochondrial membrane. The dense layers are separated by a less dense region *circa* 30 to 80 A thick which is probably continuous with the region lying between the two membranes of the mitochondrial membrane.

The double structure of the mitochondrial membrane and of the cristae divides the mitochondrion into two regions: an outer region between the parallel layers of the cristae and mitochondrial membrane; and an inner region between the cristae, that occupies most of the volume of the mito-

chondrion. A relatively homogeneous but sparsely distributed matrix is less electron-dense. The basic structure described is remarkably constant and has been found in all plants so far examined, excepting the blue-green algae (10, 47). In fact, the cristae mitochondriales are accepted as the basis for identification of mitochondria under the electron microscope.

Electron microscope observations of mitochondrial preparations show that apparently intact mitochondria can be isolated from tissues by differential centrifugation, but varying degrees of disorganization may occur—particularly through osmotic effects. When more is known about the factors required for stabilizing mitochondria in suspension, improved isolation procedures will make the preparation of intact mitochondria a routine matter (21, 24, 46, 60).

The gross chemical composition of plant and animal mitochondria is similar, as might be anticipated from the similarity in biochemical behaviour. Both are lipoprotein bodies possibly containing pentose nucleic acids. These analyses refer to mitochondrial pellets isolated by differential centrifugation and therefore variously contaminated. Future analyses should refer to preparations whose structural composition has been determined by electron microscopy (28, 31, 51).

The swelling behaviour and the reactions of mitochondria to externally applied substrates and salts demonstrate that mitochondria have permeability properties [Laties (42); Robertson *et al.* (68)]. Presumably these properties would be located in the membranes, but whether they are confined to the limiting layer of the mitochondrial membrane or to the entire membrane system including the cristae is not known. Since soluble protein is released during swelling, this material is probably held within the mitochondria by the membranes, possibly in the inner cavity between the cristae, and may account for the matrix seen in electron micrographs.

During centrifugation the structure of the mitochondria may become disorganized but the actual membranes are not destroyed. These behave as relatively solid structures that may correspond to solid lipoprotein films. Sjöstrand (74) interprets the double membrane as consisting of two protein layers enclosing a bimolecular lipid layer (corresponding to two dark lines and empty zone seen in electromicrographs).

Now that the mitochondrion can be identified both as a specific structural and a specific biochemical system, current investigations aim at a more detailed understanding of the relationship between metabolic activity and structure. These studies are following both a degradation of mitochondria into biochemical units of decreasing complexity, synthesis of mitochondrial activity by the recombination of enzymes systems, and observations on the structure of the functional units studied by electron microscopy, sedimentation, and other means.

At least four integrated enzyme systems or functional units have been identified in mitochondria: the fatty acid oxidation cycle, the citric acid

cycle, the electron transport system, and the oxidative phosphorylation system. One physiological process, the ability to accumulate salts against a gradient, has also been noted (28, 31, 51, 68). As these various activities are linked chemically it seems reasonable to assume that they may be linked structurally and that structural units corresponding to each functional unit may exist.

The probability of structural separation of the units is shown by Laties' observation that the phosphorylative system is destroyed following the swelling of mitochondria in hypotonic solutions, whereas succinate oxidation is retained (42), although such a result could be explained by the loss of soluble co-factors during swelling.

Succinate oxidation has been known to be associated with insoluble particles for many years (30). More recently Crane, Glenn & Green (30) isolated a macroparticle that appears to contain the complete electron transport system from beef heart mitochondria. They believe the particle to be a protein unit. Since the cristae and mitochondrial membranes are insoluble in aqueous media, the electron transport system is possibly located on the cristae.

If the oxidative enzymes are associated with the cristae, a correlation between the number of cristae per unit volume of mitochondria and respiratory activity might be expected. This has been demonstrated in animal tissue (60), but no data exist for plant mitochondria; nor has the intracellular localization of mitochondria in relation to cell processes been investigated in plants. In muscle the mitochondria are arranged around the I-bands of the myofibrils, and in the axon terminals of synapses. In some animal cells the mitochondria are permanently located close to an affector organ, or in regions of high energy requirement (60). A few observations on plant tissue suggest that mitochondria may be more concentrated in the companion cells than other cells (47). Phloem respiration in *Cucurbita* is tenfold greater than that in surrounding parenchyma [Duloy (20)].

Current work strongly supports the significance of structure in cell organization. The limiting membranes exercise some control by affecting the movement of substrates, co-factors, and solutes between the organelles and the cytoplasm. Integration of multi-enzyme systems could arise from an appropriate spatial distribution of enzymes in each system. While a definite pattern of enzymes can be vizualized in solid particles or in membranes, difficulties arise in extending the concept to soluble enzyme systems, which probably occur in the matrix of the mitochondria, the matrix of the chloroplast, and the matrix of the cytoplasm. The latter probably contains the glycolytic enzymes. These can be isolated as a soluble fraction capable of carrying out the glycolytic cycle *in vitro* under conditions where a structural three-dimensional organization seems improbable. An interesting suggestion (30) based on the antigen-antibody reaction indicates a way in which enzymes could be linked structurally even in solution, and suggests

that the role of the protein moiety of the enzyme may be concerned partly in forming a specific binding of enzyme to enzyme. This binding may hold the key to the organization of cellular activity. Assuming that something of this sort occurs, then structure may be the key to the organization of the cell as a living system.

Chloroplasts

Until recently there was some uncertainty about the cytological identity of chloroplasts and mitochondria [Newcomer (54); Weier & Stocking (83)]. Several lines of approach already discussed have established, beyond question, the independent existence of mitochondria and chloroplasts as more or less autonomous cytological and biochemical organelles.

The basic submicroscopic structure of the chloroplast is known and has been described in several recent review articles [Frey-Wyssling (26); Granick (29); Mühlethaler (53); Leyon (43); Mercer (48); Thomas *et al.* (79)]. The basic structure of chloroplasts is similar in all higher plants so far examined. The photosynthetic structures of certain lower forms such as blue-green algae (10, 47) and photosynthetic bacteria (47) may be different.

There is general agreement that the chloroplast is surrounded by a limiting, double-layered membrane, *circa* 100 A in thickness, separated by a narrow zone or space which is not electron-dense. Apparently the controversy about the existence of a chloroplast membrane has been resolved, at least in the higher plants. Little progress has been made in interpreting the molecular nature of the membrane. By analogy with the membrane structure proposed for mitochondria, McLean (47) points out that the chloroplast membrane could consist of two layers of protein separated by a bimolecular lipid layer. Frey-Wyssling believes that the membrane contains fibrous protein (26).

The swelling and permeability properties of chloroplasts show that the membrane is differentially permeable rather than semipermeable. Actually a semipermeable membrane is an impossibility because the chloroplast (and mitochondrion) must exchange solutes between itself and the external environment (49).

Two types of chloroplast have been described. One is the lamellate chloroplast, in which the body of the chloroplast contains numerous lamellae embedded in a matrix which may contain starch grains, dense spherical bodies, and dense irregular granules *circa* 100 A in diameter, both in the matrix and on the lamellae. This type resembles a sac containing flat plates in a matrix, and has been found in algae and higher plants [Hodge; McLean & Mercer (34); McLean (47); Mercer (48); Thomas (80)]. The second type of chloroplast contains grana interconnected by lamellae with a matrix between the intergrana (stroma) lamellae. Starch grains and dense spherical bodies are found only in the matrix. The grana consist of highly orientated lamellae, are usually cylindrical in shape, and have been likened to a

pile of pennies. This type of chloroplast resembles the first type, except for the presence of the grana.

Both types of chloroplasts contain densely staining spherical bodies, from 20 A to 0.2 μ in diameter, between the stroma (intergrana) lamellae. The orientation of the lamellae is disturbed when these bodies are large. On the basis of their spherical shape, solubility in alcohol-ether solutions, high electron density, and similarity to the carotenoid-containing bodies in the chloroplasts of the antheridial sheath of *Chara australis* (47) and in the eyespot of *Chlamydomonas* (70) and *Euglena* (84), they have been identified tentatively as a carotenoid lipid phase [McLean (47)].

The existence of lamellae in the regions between the grana raises a problem of terminology. On the basis of optical homogeneity these regions were assumed to be devoid of structure and were therefore defined by light microscopists as the stroma. Possibly the term stroma could be used in the future for the apparently homogeneous matrix lying between the lamellae, and the lamellae could be described as stroma or grana lamellae. Some investigators have described the lamellae as intergrana, others as stroma-lamellae. Sager & Palade (70) propose that the term "stroma" should be abandoned and replaced by "continuous matrix." To prevent confusion a standard terminology should be adopted.

Although there is general agreement about the basic submicroscopic structure of chloroplasts, opinions differ about the fine structure and dimensions of the lamellae and grana. One view pictures the grana as a pile of flat, hollow, membrane-bound sacs or discs supported and interleaved between lamellae 30 A thick, the latter being continuous with the stroma lamellae. The membranes of the hollow discs are about 65 A thick. The contents of the discs are believed to differ from the matrix (stroma) between the stroma lamellae in the grana; and the fine structure of the grana and stroma lamellae is believed to be different [Steinmann & Sjöstrand (77)]. It has been postulated that the grana discs are composed of globular macromolecules of lipoprotein 65 A in diameter [Frey-Wyssling (26)]. From arguments based on observations of the rather specialized chloroplast of *Chlamydomonas,* Sager & Palade (70) argue that the disc is the basic structural unit of the chloroplast and speculate about its possible role in photosynthesis.

According to another view, the structures of the grana and stroma lamellae are identical, and the grana are simply regions where the stroma lamellae have bifurcated and become more highly oriented. The lamellae as described as having a compound structure 125 A thick, consisting of a dense, granular, frequently double mid-zone, the P-zone *circa* 35 A thick; with two less dense layers, *circa* 45 A thick; (L-zones) on either side of the P-zone; and frequently with a thin dense line (C-zones) on the outer edges of the L-zone. The P-zone is assumed to be protein and the L-zone lipid, with chlorophyll on the outer surface. Chlorophyll is assumed to

occur on all lamellae, and is not localized in the grana. This type of lamellae structure has been observed in both grana and non-grana types of chloroplasts (34, 37, 48).

Differences in the interpretation of structures from electron micrographs are inevitable in a rapidly developing field where techniques are still relatively imperfect and the possibility of artifacts is ever present. Chloroplasts, like other living systems, are extremely labile and rapidly undergo disorganization in unfavourable environments. Osmotic disorganization of isolated chloroplasts is known to occur following changes in the tonicity of the suspension medium, and disorganization can also occur during fixation. Drastic treatment transforms the highly organized fine structure into a swarm of membrane-bound vesicles. With less drastic treatment the grana may inflate to give saclike discs (47 to 49). Alternatively the collapse of "discs" during fixation may give apparently solid lamellae. This possibility has not been examined experimentally. It seems conceivable that the differences between the dimensions of lamellae and between the structure of the grana which have been reported in the literature could be due to changes during fixation.

Many attempts have been made over the years to document the chloroplast in terms of chemical composition [Rabinowitch (67)]. The more common constituents include soluble and insoluble protein, lipids and phospholipids, and the pigments. The chloroplasts, like other living systems, are lipoprotein structures but are unique in that they contain pigments. Since accurate information about the pigments may supply the key to unravelling the internal structure of the chloroplast, much effort has gone into the problem of the state and distribution of the pigments in the chloroplast. Regrettably many of these data are likely to be seriously in error, possibly useless, if chloroplasts are as labile as other lipoprotein systems, such as mitochondria and the endoplasmic reticulum.

The various chlorophyll-protein complexes which have been described, and taken as evidence for the existence of a definte chlorophyll-protein structural unit within the chloroplast, have been isolated from disintegrated chloroplasts (67). By analogy with the behaviour of mitochondria and endoplasmic reticulum during fractionation, and the disorganization of structure which occurs during swelling, one might expect a spectrum of membrane-bound vesicles when chloroplasts are fragmented. Such vesicles are likely to enclose variable quantities of other chloroplast components and form a heterogeneous group which would sediment out at different speeds of centrifugation, and possibly give apparent protein-chlorophyll fractions.

Similarly, the published analytical data for grana may or may not bear an accurate relationship to grana *in situ* since it is doubtful whether the grana would maintain their integrity through the isolation procedures adopted (67). Also, chlorophyll-protein ratios based on gross analytical procedures may have little significance.

A detailed electron microscope study of chloroplasts during cell-fractionation procedures is urgently needed and is actually essential before the value of any analytical data can be assessed.

The distribution of chlorophyll and the other pigments within the chloroplast is still a controversial matter. Since the 1880's botanists have been arguing whether or not the pigments are localized in the grana. Both views are held at the present moment. For example, Frey-Wyssling (26), Thomas (80), and Sager & Palade (70) argue that chlorophyll is localized in the grana, whereas Hodge, McLean & Mercer (34) and McLean (47) favour chlorophyll being present on all lamellae surfaces.

The identification of the precise nature of the "lipid-carotenoid" droplets is important. If much of the carotenoid is associated with a lipid phase and not with chlorophyll, molecular models of the pigment system based on the gross analysis of chloroplasts would be subject to considerable error. At least some of the carotenoids must be closely associated with the chlorophylls since light energy absorbed by carotenoids is transferred to the chlorophylls and used in photosynthesis (7).

The inadequacy of the chemical data, doubt about the reality of the protein-chlorophyll complexes, the localization of the pigments in the grana, and the nature of the dense spherical bodies are serious deficiencies in our knowledge of the basic chemical structure of the chloroplast. Despite these deficiencies several models of the molecular architecture of the pigment system have been formulated [Wolken & Schwertz (86); Hodge; McLean & Mercer (34); Frey-Wyssling (26)]. These are inspired by the original suggestions of Hubert (39). Too much confidence should not be placed in the details of such models until more reliable analytical data has been obtained.

Since the swelling that occurs in the matrix has characteristics of the swelling of a hydrophilic protein system, the matrix or stroma may consist primarily of hydrophilic globular proteins (26, 49). This would account for the granular-homogeneous appearance of the matrix in electron micrographs.

The present position regarding chloroplast structure may be summarized as follows: The lamellae type of chloroplasts consists of insoluble lipoprotein lamellae associated with the pigments, embedded in the aqueous environment of a hydrophilic protein matrix or stroma, and enclosed within a differentially permeable membrane. Grana-type chloroplasts have a similar structure but are more highly differentiated into grana, stroma-lamellae, and stroma; but whether the pigments are localized in the grana has not yet been resolved.

Isolated chloroplasts can be described with considerable accuracy as biochemical entities. Whole isolated chloroplasts carry out a complete photosynthetic process with physiological characteristics similar to those of photosynthesis in intact leaves. Hence they must contain the entire enzyme machinery and co-factor complement necessary for photosynthesis. Al-

though systematic studies on the degradation of chloroplasts into simple functional units and the resynthesis of photosynthetic activity from fragments and co-factors are only in their infancy, several units of a level of biochemical complexity below that of the whole chloroplast have been identified [Arnon and co-workers (3); Frenkel (25); Hill (33)].

These biochemical units are the ones concerned with the photolysis of water, with photosynthetic phosphorylation, with CO_2-fixation, and presumably with starch synthesis. Each unit of function exists as an integrated multi-enzyme system controlling a different complex phase of photosynthesis, but chemically linked with the others to produce the overall photosynthetic process. As the existence of multi-enzyme functional units requires a high level of organization, the question arises whether such organization has a structural basis depending on the spatial relationship between enzymes, co-factors, and substrates. Comparatively little precise information exists about the internal distribution of enzymes within the chloroplast in relation to the fine structure determined by electron microscopy and other techniques, but sufficient data are available to permit interesting speculation.

The water-soluble enzymes concerned with starch synthesis appear to be localized in the matrix between the intergrana lamellae. Starch grains are always found between intergrana lamellae, never within the framework of the lamellae or in the grana, and starch grains form in chloroplasts of etiolated leaves which contain no lamellae (47, 48).

The biochemical stability of the functional units directly involved in photosynthesis fits the following sequence: CO_2-fixation is less stable than oxidative phosphorylation, which, in turn, is less stable than the photolysis of water. The Hill reaction is relatively stable, but the other two reactions may no longer be observed when chloroplasts undergo swelling. Oxidative phosphorylation, but not CO_2-fixation, can be restored in swelled chloroplasts by the addition of certain soluble co-factors: Mg^{++}, ascorbate, vitamin K; and CO_2-fixation can be restored by the addition of a chloroplast extract which contains water-soluble enzymes. This pattern suggests that CO_2-fixation requires all three units, oxidative phosphorylation two units, and photolysis of water one unit of function only [Arnon (3)].

Electron microscope studies of swollen chloroplasts show that swelling occurs in the matrix, not the lamellae, and the swelling properties of chloroplasts are consistent with the concept that the matrix is an aqueous protein-rich phase. For small degrees of swelling, the lamellae in both grana and intergrana regions separate but retain their orientation; with further swelling the intergrana lamellae fragment and the grana lamellae separate to form closed sacs; and in severe swelling all of the lamellae fragment, filling the interior of the chloroplast with innumerable hollow sacs. The electron density of the region between the lamellae decreases with increased swelling, suggesting that material is lost as the matrix becomes disorganized (49).

Correlation of these biochemical and structural observations suggests that the Hill reaction is localized on the water-insoluble lamellae and the enzymes of oxidative phosphorylation are also localized on the lamellae but with some part of the electron transfer system and co-factors located in the matrix, while the CO_2-fixation unit is most probably in the matrix along with the enzymes concerned with conversion of sugar to starch.

Fluorescence phenomena and photosynthetic efficiency in light of different wavelengths, indicate that pigment molecules are linked energetically. Light energy absorbed by the carotenoids and chlorophylls is transferred to a chlorophyll which is at the end of a chain of events (7). The existence of a photosynthetic unit involving several thousand chlorophyll molecules was postulated from kinetic studies over 20 years ago [Emerson (23)]. Evidence therefore supports the existence of a physical unit, but whether this corresponds with a unit of structure is an open question, particularly if energy transfer is by inductive resonance which operates over intermolecular distances of the order of 60 A [Duysens (22)]. Alternatively, as energy transfer would be more efficient in a highly orientated system with a high concentration of absorbing molecules, a unit of structure is feasible; but an orientated system need not be a discrete unit of structure since it could be part of a continuous structure such as a molecular film.

An apparent volume of $10^6 A^3$ has been estimated as the size of the structural unit of the Hill reaction in fragmented spinach chloroplasts. Such particles contain about 100 chlorophyll molecules [Thomas, Blaauw & Duysens (79)]. It does not follow that the hundred chlorophyll molecules and associated fragments constitute the approximate critical number of molecules of the physical unit. A slow rate of Hill reaction occurred in particles of $2 \times 10^5 = A^3$ indicating that even fewer pigment molecules may be energetically linked in a solid particle. Presumably the Hill reaction occurs in a system with a higher level of complexity than one involving just light absorption, since it involves both light absorption and enzymatic processes.

Although the separate identity of mature chloroplasts and mitochondria is established, problems about their origin and ontogeny remain unsolved. The basic problem is shown by some data for *Chara australis*. During the expansion of the internode cell the number of chloroplasts increases from a few hundred to a few million and the individual chloroplasts increase several hundredfold in volume; also the number of mitochondria increases enormously [Peebles (64)]. Clearly, the organelles have an origin and an ontogeny.

Despite the much higher resolution of the electron microscope, the origin of the organelles has not been satisfactorily explained as yet. Each of the various theories of the light microscopists finds support amongst electron microscopists (34, 37, 40, 43, 46, 53, 73).

An interesting observation is that developing mesophyll cells contain definite bodies not recognizable as either chloroplast or mitochondrion.

These bodies consist of a more or less empty sac. At a later stage of development bodies that are definitely chloroplasts and mitochondria are found. Apparently the bodies differentiate into chloroplasts and mitochondria, but the problem of the possible duality of the bodies remains (48).

Another interesting feature seen in the cytoplasm of plant cells consists of membrane-bound vesicles, apparently without internal structure, and ranging in size from 20 to 1000 A (published electron micrographs). The possibility exists that they are artifacts. Vesicle formation from cytoplasm and from mature chloroplasts and mitochondria can be experimentally caused by osmotic disorganization and faulty fixation (36). On the other hand, these may be real components of the cytoplasm. Are some of these vesicle-bodies the precursors of both the chloroplasts and mitochondria? Have they a duality of potential or are there several distinct kinds and are they elementary self-duplicating units? At the present time the sites and the mechanism of the origin of the cell organelles are not known. It has been suggested that the endoplasmic reticulum may develop from the fusion of cytoplasmic vesicles (34, 73).

The elusiveness of the solution to this problem is probably partially because of the difficulty of interpreting electron micrographs. Apparent binary fission figures can result from the angle of sectioning, and convolutions of the nuclear membrane or organelle can give the impression of budding. Serial sections may help, but even under such circumstances the difficulty of interpreting the significance of an attenuated dumb bell body still remains.

Although the initial phases in the ontogeny of the organelles are not understood, some facts are known about later stages, particularly for the chloroplast.

The smallest bodies identifiable as chloroplasts are about 0.2 μ in diameter (47). This places their dimensions beyond the limit of the light microscope and therefore casts doubt on many of the claims made by light microscopists. For example, the theory of the primary granum which explains the origin of the chloroplast and grana from the division of a primary granum can no longer be accepted.

Recent observations suggest that the development of structure in the early stages of ontogeny involves the differentiation of a crystalline-like body [Leyon (43); Heitz (40)]. Young chloroplasts in etiolated or green tissue contain a structure which, in section, bears some resemblance to a protein crystal. Lamellae appear to arise from the body and then extend throughout the chloroplast. During subsequent growth the number and area of the lamellae increase. Some observations suggest that the lamellae arise from the fusion of vesicular structures, either internally from the crystalline body or at the surface of the chloroplast in contact with the cytoplasm (34). Chloroplasts in etiolated tissue are devoid of structure, but differentiation of lamellae occurs rapidly on exposure to light, and chlorophyll increases simultaneously (47, 85). The growth of the chloroplast involves the synthesis of chlorophyll, the other photosynthetic pigments, and protein,

which raises questions about the sites of synthesis in the chloroplast and the mechanism of lamellae duplication. Does protein synthesis occur within the chloroplast, or are soluble precursors formed in the cytoplasm, undergoing polymerisation within the chloroplast? Sissakian (78) presents evidence from tracer studies which suggests protein synthesis can occur in chloroplasts.

Information about the RNA metabolism of chloroplasts is needed, particularly since the microsomes appear to be the important sites of protein synthesis (8).

Little information has been obtained about the structural mechanism of lamellae duplication, but von Wettstein (84) claims that the lamellae arise by the splitting or division of existing lamellae. This claim is not supported by others (34) who suggest that lamellae form *de novo* by the fusion of vesicular elements.

The fragmentary nature of our knowledge about the ontogeny of chloroplasts and mitochondria demonstrates the need for collaborative work between biochemists and cytologists. In addition to the multi-enzyme systems associated with photosynthesis and respiration it seems likely that the organelles may contain the multi-enzyme systems required for the synthesis of their own structure.

Electron microscopy has resulted in a new picture of the structural detail of the cytoplasm and the organelles *in vivo*. This picture must be equated with the properties of the cytoplasm and organelles *in vivo*.

Living cytoplasm has characteristic properties which are visible without treatment in the light microscope, and these are not artifacts. On the basis of non-Newtonian flow, viscosity changes, sol-gel transformation, and protoplasmic streaming, it has been assumed that cytoplasm is a hydrogel type of system—a system without structure except at a molecular level. Electron microscopy shows the existence of an intricate intracellular system of continuous membranous vesicles associated with, or immersed in, an apparently structureless matrix. The ratio of membrane vesicles to matrix varies in different cells, but as yet the "average" ratio for plant cells is not known. The system of interconnected anastomosing vesicles gives the impression of a structural circulatory system throughout the entire cytoplasm. These different, but possibly equally true pictures of cytoplasm have to be reconciled.

It is difficult to escape the conclusion that since cyclosis occurs in the living state, the intracellular structure must be continually breaking and reforming. Possibly certain regions are more stable (the Golgi apparatus or dictyosomes, and the regions in close contact with the organelles and vacuoles), but the greater part of the cytoplasm structure would appear to have a transitory existence. If, as a consequence of streaming, the endoplasmic reticulum is constantly changing, then the continuity between reticulum, nucleus, ectoplast, and dictyosomes (and tonoplast) must have a transitory existence also. We have come to accept the concept of a continu-

ous cycle of anabolism and catabolism and the existence of metabolic pools in the biochemical sense. There appears to be a structural counterpart to biochemical lability in intracellular organization. It is plausible to suppose that at any point in time cytoplasm would have a definite structure, perhaps similar to that observed under the electron micrographs. If structure has a statistical existence then many of our classical concepts of cell physiology and cell morphology may be hopelessly inadequate. Correlation of biochemical evidence both with light and electron microscope studies can and will bring about enormous advances in our understanding of organization at the intracellular level.

LITERATURE CITED

1. Agar, H. D., and Douglas, H. C., *J. Appl. Phy.*, **26,** 1393 (1955)
2. Allfrey, V., Mirsky, A. E., and Stern, H., *Advances in Protein Chem.*, **10,** 411 (1955)
3. Arnon, D. I., *Enzymes: Units of Biological Structure and Function,* 279–305 (Academic Press, Inc., New York, N.Y., 624 pp., 1956)
4. Beans, H. W., Tahmisian, T. N., and Devine, R. L., *J. Biophys. Biochem. Cytol.*, **1,** 197 (1955)
5. Bennett, H. S., *J. Biophys. Biochem. Cytol.*, **2,** No. 4, Suppl. 99 (1956)
6. Bennett, H. S., *Ann. Record,* **125,** 327 (1956)
7. Blinks, L. R., *Ann. Rev. Plant Physiol.*, **5,** 93 (1954)
8. Bonner, J., *Am. J. Botany,* **46,** 58 (1959)
9. Brachet, J., *The Nucleic Acids,* **II,** 476–519 (Academic Press, Inc., New York, N.Y., 576 pp., 1955)
10. Bradfield, J. G., *6th Symposium Soc. Gen. Microbiol. Bacterial Anat.*, 296–317 (Cambridge University Press, New York, N.Y., 360 pp., 1956)
11. Briggs, G. E., and Robertson, R. N., *Ann. Rev. Plant Physiol.*, **8,** 1 (1956)
12. Buvat, R., *Ann. sci. nat., Botan.*, **11,** 123 (1958)
13. Caspersson, T., and Schultz, J., *Nature,* **143,** 602 (1939)
14. Chambers, R., and Hofler, K., *Protoplasma,* **12,** 338 (1931)
15. Chao, Fu-Chuan, and Schachman, H. K., *Arch. Biochem. Biophys.*, **61,** 220 (1956)
16. Chayen, J., and Fitton Jackson, S., *Mitochondria and Other Cytoplasmic Inclusions,* 134–147 (Cambridge University Press, New York, N.Y., 198 pp., 1956)
17. Clark, Peggy, *Water Relations of Protoplasts* (Master's thesis, University of Sydney, Sydney, Australia, 1955)
18. Danielson, C. E., *Ann. Rev. Plant Physiol.*, **7,** 215 (1956)
19. Davson, H., and Danielli, J. F., *The Permeability of Natural Membranes,* 2nd ed. (Cambridge University Press, New York, N.Y., 365 pp., 1952)
20. Duloy, M., *Physiology of the Phloem* (Doctoral dissertation, University of Sydney, Sydney, Australia, 1959)
21. Duve, C. de., *Mitochondria and Cytoplasmic Inclusions,* 50–61 (Cambridge University Press, New York, N.Y., 198 pp., 1956)
22. Duysens, L. N. M., *Ann. Rev. Plant Physiol.*, **7,** 25 (1956)
23. Emerson, R., and Arnold, W., *J. Gen. Physiol.*, **15,** 391 (1932)

24. Farrant, J. L., Potter, Coralie, Robertson, R. N., and Wilkins, M. J., *Australian J. Botany*, **4** (2), 117 (1956)
25. Frenkel, A. W., *J. Am. Chem. Soc.*, **76**, 5568 (1954)
26. Frey-Wyssling, A., *Macromolecules in Cell Structure* (Harvard University Press, Cambridge, Mass., 112 pp., 1957)
27. Frey-Wyssling, A., *Submicroscopic Morphology of Protoplasm* (Elsevier Press Inc., Amsterdam, Holland, 255 pp., 1953)
28. Godard, D., and Stafford, H., *Ann. Rev. Plant Physiol.* **5**, 115 (1954)
29. Granick, S., *Encyclopedia of Plant Physiol.*, **1**, 507–564 (Ruhland W., Ed., Springer-Verlag, Berlin, Germany, 850 pp., 1955)
30. Green, D. E., *Mitochondria and Other Cytoplasmic Inclusions*, 30–49 (Cambridge University Press, New York, N.Y., 190 pp., 1956)
31. Hackett, D. P., *Intern. Rev. Cytol.*, **4**, 143 (1954)
32. Hackett, D. P., *Cytologia*, **23**, 86 (1958)
33. Hill, R., *Proc. Roy. Soc. (London)*, **127**, 192 (1939)
34. Hodge, A. J., McLean, J. D., and Mercer, F. V., *J. Biophys. Biochem. Cytol.*, **2**, No. 5, 597 (1956)
35. Hodge, A. J., Martin, E. M., and Morton, R. K., *J. Biophys. Biochem. Cytol.*, **3**, 61 (1957)
36. Hodge, A. J., *J. Biophys. Biochem.*, **2**, 221 (1956)
37. Hoffmann, H., and Grigg, G. W., *Exptl. Cell Research*, **15**, 118 (1958)
38. Howard, F. D., and Yamaguchi, M., *Plant Physiol.*, **32** (5), 418 (1958)
39. Hubert, B., *Photosynthesis*, **I** (Interscience Publishers Inc., New York, N.Y., 1956)
40. Heitz, E., *Exptl. Cell Research*, **7**, 606 (1954)
41. Huff, E. L., Hogeboom, G. H., and Dalton, A. J., *J. Biophys. Biochem. Cytol.*, **4**, 87 (1958)
42. Laties, G. G., *Plant Physiol.*, **28**, 557 (1953)
43. Leyon, H., *Svensk Kem. Tidskr.*, **68**, 70 (1956)
44. Linet, N., and Brachet, J., *Biochim. et Biophys. Acta.*, **7**, 607 (1951)
45. Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnik, P. C., *J. Biol. Chem.*, **217**, 111 (1955)
46. Lund, H. A., Vatter, A. B., and Hanson, J. B., *J. Biophys. Biochem. Cytol.*, **4**, 87 (1958)
47. McLean, J. D., *The Structure of the Plant Cell* (Doctoral dissertation, University of Sydney, Sydney, Australia, 1956)
48. Mercer, F. V., *Proc. Linnean Soc. N.S.Wales*, **1**, 4 (1956)
49. Mercer, F. V., Hodge, A. J., Hope, A. B., and McLean, J. D., *Australian J. Biol. Sci.*, **8**, 1 (1955)
50. Mercer, F. V., *The Water Relations of Plant Cells* (Doctoral dissertation, University of Cambridge, Cambridge, England, 1950)
51. Millerd, A., *Encyclopedia of Plant Physiol.*, **II**, 573–83 (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, 1955)
52. Millerd, A. and Bonner, J., *J. Histochem. and Cytochem.*, **1**, 254 (1953)
53. Mühlethaler, K., *Intern. Rev. Cytol.*, **4**, 197 (1955)
54. Newcomer, E., *Botan. Rev.*, **17**, 53 (1951)
55. Novikoff, A. B., *Mitochondria and Other Cytoplasmic Inclusions*, 92–109 (Cambridge University Press, New York, N.Y., 198 pp., 1956)

56. *N.Y.S.E.M. Index* (Published quarterly) New York Society of Electron Microscopy, New York, N.Y.
57. Palade, G. E., and Porter, K. R., *J. Exptl. Med.*, **100**, 641 (1954)
58. Palade, G. E., *J. Biophys. Biochem. Cytol.*, **1**, No. 1, 59 (1955)
59. Palade, G. E., *J. Biophys. Biochem. Cytol.*, **2**, No. 4, Suppl. 85 (1956)
60. Palade, G. E., *Enzyme Units of Biological Structure and Function*, 185–215 (Symposium) (Academic Press, Inc., New York, N.Y., 624 pp., 1956)
61. Palade, G. E., and Siekevitz, P., *J. Biophys. Biochem. Cytol.*, **2**, 171 (1956)
62. Palade, G. E., *J. Biophys. Biochem. Cytol.*, **2**, 417 (1956)
63. Pease, D. C., *Anat. Record*, **121**, 723 (1954)
64. Peebles, M. J., *Studies in Cell Development in Chara australis* (Doctoral thesis, University of Sydney, Sydney, Australia, 1957)
65. Porter, K. R., Claude, A. and Fulham, E. F., *J. Exptl. Med.*, **81**, 233 (1945)
66. Porter, K. R., *J. Exptl. Med.*, **97**, 727 (1953)
67. Rabinowitch, E. I., *Photosynthesis*, **I** (Interscience Publishers Inc., New York, N.Y., 599 pp., 1956)
68. Robertson, R. N., Wilkins, M. J., Hope, A. B., and Nestel, L., *Australian J. Biol. Sci.*, **8**, 164 (1955)
69. Sager, R., and Palade, G. E., *Exptl. Cell. Research*, **7**, 584 (1954)
70. Sager, R., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **3**, 463 (1957)
71. Schachman, H. K., *Arch. Biochem. Biophys.*, **38**, 245 (1952)
72. Sedar, A. W., and Porter, K. R., *J. Biophys. Biochem. Cytol.*, **1**, 583 (1955)
73. Sitte, P., *Protoplasma*, **49**, 447 (1958)
74. Sjöstrand, F. S., *Proc. Intern. Conf. Electron Microscopy*, 26–37 (Roy. Microscopial Soc., London, England, 705 pp., 1954)
75. Sjöstrand, F. S., *Physical Techniques in Biological Research*, **III**, 241–98 (Academic Press, Inc., New York, N.Y., 728 pp., 1956)
76. Stanley, R. G., and Conn, E. E., *Plant Physiol.*, **32**(5), 412 (1957)
77. Steinmann, E., and Sjöstrand, F. S., *Exptl. Cell Research*, **8**, 15 (1955)
78. Sissakian, N. M., *Advances in Enzymol.*, **20**, 201 (1958)
79. Thomas, J. B., Blaauw, O. H., and Duysens, L. N. M., *Biochim. et Biophys. Acta*, **13**, 20 (1954)
80. Thomas, J. B., *Endeavour*, **17**, No. 67, 156 (1958)
81. Tso, P. O. P., *J. Biophys. Biochem. Cytol.*, **2**, 451 (1956)
82. Watson, M. L., *J. Biophys. Biochem. Cytol.*, **1**, 257 (1955)
83. Weier, T. E., and Stocking, C. R., *Botan. Rev.*, **17**, 14 (1952)
84. Wettstein, D. von, *Heriditas*, **43**, 303 (1957)
85. Wolken, J. J., and Palade, G. E., *Ann. N.Y. Acad. Sci.*, **56**, 873 (1953)
86. Wolken, J. J., and Schwertz, F. A., *J. Gen. Physiol.*, **37**, 111 (1953)

NITROGEN FIXATION[1,2]

By Conrad S. Yocum

Department of Botany, New York State College of Agriculture, Cornell University, Ithaca, N.Y.

Introduction

1957 marked the 100th anniversary of Lawes, Gilbert, and Pugh's pioneer experiments in biological nitrogen fixation (1). This century of progress in many directions and to various depths has produced a voluminous literature, only a small fraction of which is surveyed here. In order to avoid repetition and to keep the review brief, the author has limited the coverage, wherever possible, to the more recent publications that have not been previously summarized. For example, the evidence for ammonia as an intermediate is fully documented elsewhere and is not repeated. Neither has any attempt been made to include a general discussion of related topics such as hydrogenase or the respiratory processes of nitrogen-fixing organisms. However, a number of excellent reviews are available and they may be consulted for a more extensive survey. Wilson recently gave an historical account of the early discoveries (1) and reviewed the nonsymbiotic fixation (2). The symbiotic fixation, hardly touched in the present paper, is extensively covered by Allen & Allen (3), Nutman (4), and Shields (5). Jensen has reviewed the family *Azotobacteriaceae* (6), and Fogg has reviewed the photosynthetic nitrogen-fixing microorganisms (7). Virtanen's 1955 review (8) is generally not as available as is Virtanen & Rautanen's 1952 summary (9). The most recent reviews are those of Nicholas (10), Bond (11, 12), and Leaf (13). A number of articles, some of which are referred to individually, dealing with N_2 fixation and related topics are included in: *Biochemistry of Nitrogen,* a collection dedicated to Virtanen (14); *Inorganic Nitrogen Metabolism,* edited by McElroy & Glass (15); and *Nutrition of the Legumes,* edited by Hallsworth (16).

Organisms and Ecology

In any field of plant physiology, the progress at some stage is advanced by the discovery of new organisms or unique properties of previously described species. For this reason, brief consideration is given to the important topic of newly described organisms. Wilson lists a number of nonsymbiotic microorganisms (2), Fogg discusses the blue-green algae and photosynthetic bacteria (7), Allen & Allen consider the legume symbionts (3), and Bond reviews the non-legume symbionts in higher plants and a

[1] The survey of literature pertaining to this review was concluded November 1959.

[2] The following abbreviations will be used: DPN (diphosphopyridine nucleotide); DPNH (diphosphopyridine nucleotide, reduced form); PCA (3,4-dihydropyridazinone-5-carboxylic acid); Pi [inorganic(ortho)phosphate]; TPNH (triphosphopyridine nucleotide, reduced form).

variety of old and new agents that have recently been tested with modern critical methods (11). Some organisms not included in the above reviews and for which positive evidence of nitrogen fixation has been published are listed here.

Organism	Author(s)	Reference
1. Nonsymbiotic		
Achromobacter sp.	Jensen	(17)
	Proctor & Wilson	(18)
Bacillus sp.	Hino & Wilson	(19)
Gram negative coccus	Roy & Mukherjee	(20)
Nocardia calcarea	Metcalfe & Brown	(21)
Pseudomonas sp.	Proctor & Wilson	(18)
Pullularia sp.	Brown & Metcalfe	(22)
Yeast	Nemeth	(23)
2. Symbiotic		
Coriaria arborea	Stephenson	(24)

With the exception of legumes under cultivation (3), the quantitative role of each of the multitude of nitrogen-fixing agents in nature is difficult to assess (6). Some recent laboratory trials suggest, however, that the non-legume symbionts fix with efficiencies approaching [Stephenson (24)] or surpassing [Bond (11)] those of legumes. Nonsymbiotic fixation by unidentified soil organisms [Delwiche & Wijler (25)] in "inverted lawn" fixed 12 lbs. N/acre/31 days. Of a variety of factors tested, the addition of soluble carbohydrates increased the rate of fixation in soil to the greatest extent. For example the addition of 780 mg. sucrose per 10 gm. of soil led to a fixation equivalent to 344 lbs. N/acre/31 days. Dugdale, *et al.* (26) added $^{15}N_2$ to lake water and obtained measurable fixation rates but it is not yet possible to evaluate the ecological significance of fixation in natural waters.

Culture Requirements

A prerequisite to physiological and biochemical investigations of nitrogen fixation is the growth of test organisms under reproducible and optimum culture conditions. Ideally the observed growth rates should approach or equal the genetically controlled values; i.e., they should not be limited by either concentration or rate of supply of any component of the culture medium. Under such conditions it is then possible to experimentally control the rate of nitrogen fixation and measure it simply by the growth rate of the cells. There appear to have been relatively few studies of nitrogen-fixing organisms in which the primary purpose was to obtain these requirements. Conditions for obtaining large cell populations have been described recently, however. Alexander & Wilson (27) obtained high yields of *Azotobacter vinelandii* cells per unit of culture medium by increasing the sugar content of Burk's medium (plus 0.1 mg. Mo/l.) to 6 to 8 per cent. A high rate of

oxygen supply and 8 mg. Fe/l. were also required. Both the total yield (1,000 μg. N/ml.), and the growth rate (approximately 0.26 per hr.), were independent of the singularly added microelements: copper, zinc, cobalt, manganese, and boron. The exceptionally large iron requirement for *Azotobacter* and a neutral (pH 7 to 8) medium are factors that favor precipitation during autoclaving of culture media. Such precipitation is readily prevented by addition of ethylenediamine tetraacetate [Hutner *et al.* (28)] or similar chelating agents, but few investigators have recognized the usefulness of chelation in the culture of nitrogen-fixing organisms. Environmental factors leading to a yield of 7 to 8 gm./l. (dry weight) of *Anabaena cylindrica* growing on molecular nitrogen have been enumerated by Allen & Arnon (29).

The significance of molybdenum in the metabolism of inorganic nitrogen in plants has received much and due attention in recent years. Evans (30), Nicholas (31) Hewitt (32), and Nason (33) have prepared general reviews and a large fraction of the McCollum Pratt symposium *Inorganic Nitrogen Metabolism* (15) was devoted to molybdenum. Now that the requirement for Mo by *A. vinelandii* has been established [Esposito (34), Esposito & Wilson (35)], the essentiality of this element for nitrogen fixation appears to be general among nitrogen-fixing agents. However, the quantities of Mo supporting half the maximum total fixation differed quite markedly from the previously reported values of Mulder (36) for *A. chroococcum.* About 100 μg./l. Mo were needed to half saturate *A. chroococcum* while about 1 μg./l. Mo half saturated *A. vinelandii.* Growth on NH_4^+ was independent of added Mo in both species and about 6 μg./l. effectively half saturated *A. chroococcum* growing on NO_3^-. Takahashi & Nason (37) and Keeler & Varner (38) demonstrated a clear molybdenum-tungsten competition by showing that Mo requirements of *Azotobacter* increase in the presence of added W. On the contrary, Davies & Stockdill (39), in field trials with nodulated white clover obtained evidence for W replacing Mo. W at 4 oz./acre produced increases in yields equal to those resulting from the application of 2.5 oz. of Mo/acre. Perhaps W competed with Mo for binding sites on the soil particles, thus exchanging with Mo, and releasing enough Mo from the soil to meet the plant requirements.

The calcium nutrition of nitrogen-fixing agents has been studied by several investigators. According to Esposito & Wilson (35), Ca is required for *Azotobacter* growth on molecular N but not on combined N; Norris & Jensen (40) found Ca to be essential for growth on all N sources. Norris and Jensen attributed the Esposito and Wilson result to a large inoculum. Ca is indispensable for *Anabaena cylindrica* grown with molecular N and NO_3^- [Allen & Arnon (29)]. *Nostoc muscorum* grew optimally at 0.1 mg./l. added boron [Eyster (41)], and *A. chroococcum* yielded similar results when cultured on molecular N [Gerretsen & deHoop (42)]. A cobalt requirement for blue-green algae, grown on all forms of N, has been demonstrated by Holm-Hansen *et al.* (43) and a similar requirement for legume nodules and *Rhizobia* in pure culture follows from their vitamin B_{12} content

[Levin *et al.* (44)]. Apparently, manganese, copper, and zinc have not been shown to be essential for any nitrogen-fixing organisms except legumes, but it is recommended that they also be added to culture media, especially those containing chelating agents.

Synthetic media for the culture of anaerobic, nitrogen-fixing *Clostridia* must be supplemented with one or more growth factors. Virtanen and Lundbom's strain of *Cl. pasteurianum* requires folic acid (45); Carnahan & Castle's strain (46) and *Cl. kluyveri* [Bornstein & Barker (47)] require biotin and *p*-amino benzoic acid. The biotin and Fe requirements of *Cl. pasteurianum* were greater during fixation than during NH_4^+ assimilation (46).

The general cultural conditions of symbiotic species and nodulated legumes in the field have been discussed elsewhere [Allen & Allen (3) and Anderson (48)]. Nodulation of a high proportion of excised bean roots in pure culture was reported by Raggio, Raggio & Torrey (49). Their success was based on the ingenious method of supplying a carbon source and growth factors exclusively to the base of the root. Minerals and *Rhizobia* were supplied to the lateral and root hair surfaces in the usual manner. Thus, cultural conditions are favorable only for the growth of those bacteria that infect the roots. No tests of nitrogen fixation were made on these nodulated excised roots. Bergersen's recent observation (50) that soybean nodules fix nitrogen only two weeks after development has apparently been overlooked for many years and may account, in part, for the variable rates of fixation by excised nodules and by nodule extracts.

Inhibitors

With the possible exception of some optical properties and those properties measurable in an intact organism or system, little can yet be said of nitrogen-fixing enzymes. Of the latter properties, their behavior in the presence of certain inhibitors has been significant in nitrogen fixation, as it has in the fields of respiration and photosynthesis. Although much of our knowledge of substances inhibitory to fixation is well established [Wilson (2) Virtanen (8)], some recent additions to the literature may give more unity to the topic and are discussed below.

Mozen *et al.* (51) have reinvestigated the effect of N_2O on *A. vinelandii*. N_2O inhibited the growth rate on N_2 but not on NO_3^- or NH_4^+; these results were confirmed with rate of ^{15}N uptake measurements. The previously reported inhibition of growth on NO_3^- by N_2O may have been due to experimental conditions that did not preclude fixation. Several groups of investigators have studied the hydrogen inhibition of anaerobic fixation. Hiai *et al.* (52), using an organism, later identified as probably a species of *Aerobacillus* (19), estimated the Michaelis constant (K_m) for fixation to be 0.03 atm. N_2. Hydrogen inhibited growth via nitrogen fixation but not via NH_4^+ assimilation. This inhibition was competitive; an H_2/N_2 ratio of about 3 was required for 50 per cent inhibition. Oxygen also inhibited and Hino (53) had previously shown sensitivity to N_2O and CO. Hydrogen

inhibition of the anaerobic N_2 fixation by *Rhodospirillum rubrum* has been investigated by Pratt & Frenkel (54) using a mass spectrometer. Inhibition was reversible but no studies were made to determine whether it was competitive. They also showed the requirement of light and anaerobic conditions for N_2 uptake. The anaerobic N_2 fixation by *Aerobacter aerogenes* is inhibited by molecular hydrogen according to Pengra & Wilson (55). These rather similar results with three anaerobic agents differ from those of Rosenblum & Wilson (56) who obtained small and inconsistent suppression of fixation with H_2 by *Cl. pasteurianum*. It seems quite likely that consistent hydrogen inhibition of nitrogen fixation by *Cl. pasteurianum* may be demonstrated under certain conditions, e.g., several atmospheres pressure H_2. If such a supposition is correct, hydrogen inhibition is a general characteristic of biological N_2 fixation, and the *Clostridium* system is less sensitive than others reported in the literature.

Oxygen not only inhibits the anaerobic fixations, as shown above, but also aerobic fixations in the symbiotic and free-living forms. With soybean nodule slices [Burris, Magee & Bach (57)] maximum fixation rates were observed at about 50 per cent O_2. Higher concentrations suppressed the rate, decreasing it to zero at 90 per cent O_2. Half saturation required about 20 per cent O_2 and 2.5 per cent N_2.

Parker & Scutt (58) have studied the oxygen inhibition in *A. vinelandii* by manometric methods. At 10 per cent O_2 half the maximum rate of fixation required 1 per cent N_2 but at 20 per cent O_2, 2 per cent N_2 was required. They concluded that oxygen competes with nitrogen for reductant. In addition to the specific competitive inhibition, a nonspecific one occurred "whether nitrogen fixation is proceeding or not." The results of Parker & Scutt are in agreement with those of Wilson & Roberts (59) who found that the K_m (Michaelis constant) for *A. vinelandii* fixation at 20 per cent O_2 was 2 per cent N_2 and 6.6 per cent N_2 at 30 per cent O_2.

From the recent experimental evidence cited above and the more extensive older literature, the following characteristics appear to be general. Nitrogen fixation requires 1 to 2 per cent N_2 for half saturation, and it is specifically and competitively inhibited by H_2, N_2O, and possibly O_2 and CO at low concentrations. CO also inhibits noncompetitively and neither CO nor O_2 at high concentrations inhibit N_2 fixation exclusively.

These properties, along with the nature of reactants and end products, must be taken into account for descriptions of enzymes that combine with and reduce molecular nitrogen. A number of other inhibitors have been studied but none of these appears to be specific enough to aid in understanding the mechanism.

Hydroxylamine

Although hydroxylamine has the characteristics required of a metabolic intermediate in the reduction of nitrate to ammonia [Taniguchi *et al.* (60)] and in the oxidation of ammonia to nitrate [Hofman & Lees (61)], it appears to have few, if any, required properties of an intermediate in nitrogen

fixation. The enzyme hydroxylamine reductase, which catalyses the reaction $NH_2OH + 2\ [H] \rightarrow NH_3 + H_2O$, has been studied in *Azotobacter* grown on various forms of nitrogen. Suzuki & Suzuki (62), and Spencer, Takahashi & Nason (63) observed a two- to fourfold higher enzyme activity in NO^-_3-grown cells than in those cultured on NH_4^+ or N_2. Both of these groups concluded that hydroxylamine reductase functions in nitrate assimilation and not in nitrogen fixation. Saris & Virtanen (64) in a study of bound-hydroxylamine compounds conclude, "Since hydroxylamine compounds are formed in our *Azotobacter* cultures from NH_4-N as rapidly as from N_2 and identical compounds seem to be in question, there is no evidence any longer of an oxidative step in aerobic N_2 fixation." It may be concluded from these experiments, and those previously reviewed [Wilson (2) and Rautanen (65)], that hydroxylamine still lacks experimental support for a possible role in the fixation process.

Hydrazine

The most critical testing of hydrazine as an intermediate in biological nitrogen fixation has been published by Bach (66) and Bach & Burris (67). Hydrazine added to *A. vinelandii* grown on molecular nitrogen disappeared from non-toxic concentrations. More than one-half of the added hydrazine was recovered by acid hydrolysis of organic azines. Ammonia inhibited the rate of hydrazine disappearance. The organic azines so formed were chromatographically indistinguishable from those prepared by the nonenzymatic reaction between hydrazine and α-ketoglutaric acid, a reaction that proceeds smoothly in dilute neutral solution. The azines extracted from field-grown soybean nodules were similar. One of these azines, nonbiologically produced, had been previously identified as 3,4-dihydropyridazinone-5-carboxylic acid (PCA).

Also, the distribution of ^{15}N was compared in *Azotobacter* suspensions exposed to $^{15}N_2$ and $^{15}NH_4^+$. After the soluble components were extracted in hot ethanol (final concentration 70 per cent) they were chromatographically separated and the atom per cent ^{15}N excess over the natural abundance was determined with a ratio type mass spectrometer. Marked differences in the ^{15}N labeling of organic acids resulted. For example, PCA of cells exposed to $^{15}N_2$ contained 300 times as much ^{15}N as that from cells exposed to $^{15}NH_4^+$. Two other organic acid components, presumably related to PCA, showed rather similar ratios. On the other hand, very small differences were observed in the glutamate and aspartate labeling by $^{15}N_2$ and by $^{15}NH_4^+$. In the cells exposed to $^{15}N_2$, PCA contained three times as much ^{15}N excess as did NH_3, but in the NH_4^+ exposed cells it contained less than 0.006 times as much ^{15}N as did NH_3. Thus, these azines are characteristic of intermediates in nitrogen fixation and not of those in ammonia assimilation.

Hydrazine and PCA enriched with ^{15}N were then fed to *A. vinelandii* cells but the results of these experiments were not consistent with the $^{15}N_2$ feeding experiments. The amount of ^{15}N that was incorporated was not significantly greater than in the controls to which PCA ^{15}N was added just

prior to inactivation of the cells. Such a result may have been partially due to an excess of hydrazine, 0.63 m*M* in this experiment. In other experiments concentrations above 0.23 m*M* inhibited the disappearance of hydrazine from the bacterial suspensions. The reviewer feels justified in discarding the results of this experiment. If such procedure is acceptable, the previously mentioned isotope-distribution experiments demonstrating a higher ^{15}N content in PCA than in ammonia following exposure of cells to $^{15}N_2$, are consistent with a fixation pathway from molecular nitrogen to hydrazine to PCA to ammonia.

According to Bach the intermediates between PCA and ammonia could include glutamic acid and possibly glutamine. The relative ^{15}N concentration in $^{15}N_2$ exposed cells was: PCA and related organic acids > glutamate > ammonia. Glutamine was not included in the assay.

Bulen & Doughty (68) showed that externally supplied PCA was not metabolized by intact *A. vinclandii,* but that ^{14}C-labeled PCA was converted by anaerobic cell free extracts to acids tentatively identified as α-ketoglutaric and succinic. Both of these are predicted from the above postulated intermediates, glutamine and glutamate. A comparison of Bach's results with those of other Wisconsin investigators, reviewed by Wilson (2) and Burris (69) and including Allison and Burris' short-time kinetic studies (70), is difficult. Bach isolated only those N-containing compounds soluble in hot 70 per cent ethanol, while the others emphasized the $^{15}N/^{14}N$ ratios in components of acid hydrolysates. As has been pointed out by both Burris (69) and Wilson (71), ammonia from such hydrolysates contains a mixture of "free" and "amide" NH_3. For similar reasons, isolated glutamatic acid may derive from glutamine or glutamate. Thus, a higher $^{15}N/^{14}N$ ratio in glutamate than in NH_3 may be interpreted in two ways: (*a*) glutamate precedes NH_3 during fixation or (*b*) NH_3 is the precursor but was diluted to a greater extent with labile $^{14}NH_3$, such as amide, during isolation and identification. Further comment on possible intermediates between PCA and NH_3 should probably be reserved until more specific separatory and analytical procedures are tested and applied to the problem. Some limitations of chromatography and related techniques have been enumerated by Thompson, Morris & Gering (72) and Krebs has developed a quantitative enzymatic method of high specificity for glutamate and glutamine (73). The above remarks on isotope dilution may also apply, in part, to the experiments of Leaf, Gardner & Bond (74) in which alder nodules fixed $^{15}N_2$. The atom per cent excess of ^{15}N in dilute acid extracts after a 3-hour exposure was: citrulline (carbamyl) > glutamate > NH_3.

Nodule Hemoglobin and *Azotobacter* Pigments

Ever since the recognition of the red nodule pigment as a hemoprotein its function has tantalized the students of N_2 fixation. This nodule hemoglobin has spectral properties similar to those of blood hemoglobin, yet appears not to serve as an oxygen carrier within the nodule. The evidence for such a view is twofold. The oxygen affinity of isolated soybean nodule hemoglobin is of the same order of magnitude [Keilin & Wang (75)] as that

of the respiratory enzymes, and these two pigments may be expected to compete for oxygen. Five per cent CO, which does not suppress nodule respiration, combines with > 99 per cent of the isolated pigment to form carboxyhemoglobin [Smith (76)]. However, this same concentration of CO is about 100 times that required to strongly inhibit N_2 fixation in nodulated legumes. These observations, along with the strong correlation between the hemoglobin content of nodules and the rate of N_2 fixation, suggest that hemoglobin participates in the fixation process [Keilin & Smith (77) and Virtanen *et al.* (78)]. Such a suggestion is supported by some spectrophotometric and enzymatic properties of soybean nodule extracts [Hamilton, Shug & Wilson (79)].

Hamilton *et al.* obtained difference spectra from these extracts by equilibrating them with oxygen-free helium, hydrogen, and nitrogen. Hydrogen minus helium difference spectra showed absorption maxima and minima at wavelengths corresponding to hemoglobin (Fe^{++}) minus hemiglobin (= methemoglobin, Fe^{+++}). Nitrogen minus helium spectra corresponded to a hemiglobin minus hemoglobin difference spectrum. Hamilton *et al.* suggested that hemoglobin functioned in N_2 fixation by undergoing oxidation and reduction. The fate of the N_2, which presumably was reduced concomitantly with oxidation of hemoglobin, was not determined. These experiments and the results differ from those of Keilin & Smith (77) who made visual spectroscopic observations of nodule slices in air and detected no hemiglobin via the fluoride test. In extracting and fractionating soybean heme proteins, Thorogood (80) observed, "In a fresh preparation, little (and ideally, no) oxidized pigment is present." If hemoglobin in nodules is oxidized to hemiglobin during fixation, measurable quantities of the oxidized form should be present unless the equilibrium is extremely far toward the reduced state.

Resolution of the apparent discrepancies mentioned above will require further spectral studies of nodular pigments. *In vivo* studies are especially needed for several reasons. (*a*) Extraction processes may dilute enzymes, cofactors, and soluble intermediates to the extent of interrupting steady states of N_2 fixation. (*b*) Certain enzymes (phenol and ascorbic oxidases, for example) that are features of soluble extracts do not meet the criteria of oxidases in the organisms from which they were extracted [Bonner (81), Yocum & Hackett (82)]. In this category one may place the nitrate reductase of nodule extracts [Cheniae & Evans (83)] until NO_3^- *per se*, is shown to be an intermediate in N_2 fixation.

Hamilton *et al.* also made difference spectra with cell-free extracts from *A. vinelandii*. The hydrogen minus oxygen spectrum showed maxima and minima characteristic of the flavins and respiratory cytochromes of *Azotobacter*. When nitrogen replaced oxygen, flavin and cytochrome-*b* were oxidized. As in the hemoglobin experiments, the fate of the nitrogen was not determined. Had nitrogen or some N-containing intermediate served as a terminal electron acceptor from cytochrome-*b*, one might expect a reduced product, ammonia for example, to accumulate in stoichiometric quantities.

Nitrogen Fixing Phosphorylation

When *Clostridium pasteurianum* fixes molecular nitrogen, a relatively large amount of the fixed nitrogen may be excreted by actively dividing cells to the external culture medium [Rosenblum & Wilson (56)]. For example, in a 24-hour culture that had fixed 19 μg. of nitrogen per ml. the increase of ammonia external to the cells was 6 μg. per ml. At first sight this may appear wasteful in that a substance necessary for cell synthesis is also excreted to the culture medium. However, considering N as an hydrogen acceptor and reviewing some aspects of nitrogen fixation mechanisms and glutamine metabolism, one can postulate that such an excretion of ammonia may be accompanied by a net accumulation of high energy phosphorus compounds within the cells. Thus N would be a terminal electron acceptor analogous to oxygen in oxidative phosphorylation. The following assumptions are required: (*a*) the hydrazine and α-ketoglutarate → glutamine proposed for *A. vinelandii* by Bach (67) also functions in *Cl. pasteurianum*, (*b*) glutamine, by way of a reversible glutamine synthetase [Elliot (84)] yields glutamic acid, ammonia, and ATP, and (*c*) reduction of nitrogen does not require ATP.

Assumption (*a*) is consistent with the observed similarity of labeling of the nitrogen compounds of *Clostridium* to those of *Azotobacter* [Wilson (2)]. For example, the ^{15}N content of glutamate exceeded that of the cells in both cases. Also required is the hydrazine acceptor, α-ketoglutarate. Although reactions producing α-ketoglutarate, and not requiring a citric acid cycle characteristic of aerobic organisms, have not been established for *Clostridium*, a pathway from pentose meeting these requirements has been described by Weimberg & Doudoroff, in *Pseudomonas* (85). An α-ketoglutarate-synthetic system would also be essential for any anaerobic organism when growing on ammonia, provided that glutamic acid is synthesized via glutamic dehydrogenase. Some evidence for α-ketoglutarate functioning in *Clostridium* fixation has been provided from cell free extracts [Hoch & Westlake (86)].

Little special comment is required for (*b*). Further reaction of glutamate to yield NH_3, TPNH or DPNH, and α-ketoglutarate is well known and may complete the cycle but would not lead to any net storage of a compound such as ATP within the cells.

Assumption (*c*), admittedly, is supported by little or no direct experimental evidence but some indirect arguments follow. Wilson & Burris (87) have calculated the change in free energy, ΔF, for the reduction of 0.5 N_2 with 3[H] at the reduction level of carbohydrate. Making the plausible assumptions pH = 7, $pN_2 = 0.8$ atm., and $NH_4^+ = 10^{-4}M$, the change in free energy was computed to be +17.5 kcal. A value of +14 kcal. for the same reaction has been more recently computed by Bayliss (88). From these data the oxidation-reduction potential of the 0.5 N_2-NH^+_4 system was calculated to be −0.20 volt (87). This potential is more positive than the −0.32 volt of the pyridine nucleotide couple. Hence, DPNH or TPNH are calculated to be strong enough reducing agents for the overall reduction of nitrogen to

ammonia, and the assumption (*c*) is not thermodynamically impossible. However, Bayliss (88) points out that the nitrogen-hydrazine reaction is slightly less favorable. This may not be serious if an enzyme-hydrazine complex rather than free hydrazine is an intermediate or if [H] at the reduction level of molecular hydrogen (−0.42 volt) is the reductant and formed from CH_2O without ATP expenditure. The latter is more probable in view of the evidence for a close relation between hydrogenase and N_2 fixation [Hoch, Little & Burris (89)]. Of the hydrogenase reactions thus far studied, none appear to require ATP for hydrogen evolution [Gest (90) and Packer & Vishniac (91)]. In fact, when pyruvate and orthophosphate yield molecular hydrogen and carbon dioxide, a high energy bond is formed in acetyl phosphate [Koepsell, Johnson & Meek (92)]. That hydrogenase transfers hydrogen to nitrogen has been proposed by Wilson & Burris (87) and Winfield (93).

The pertinent reactions in this section are summarized as follows:

$$N_2 + \alpha\text{-ketoglutarate} + 4[H] \rightarrow PCA$$
$$\text{glutamine} \leftarrow PCA + 4[H]$$
$$ADP + Pi + \text{glutamine} \rightleftarrows \text{glutamate} + ATP + NH_3$$
$$2[H] + NH_3 + \alpha\text{-ketoglutarate} \rightleftarrows \text{glutamate}$$

$$N_2 + 6[H] + ADP + Pi \rightarrow 2NH_3 + ATP : \Delta F = +18 \text{ to } 25 \text{ kcal.}$$

In the overall reaction, the P/e ratio (phosphate esterified per electron transferred) is 0.17. Although this P/e ratio is relatively small compared to that of most aerobic organisms (P/e = 1.5) the ratio is large compared to that of an anaerobe in which ATP is synthesized in the steps between glucose and pyruvate (P/e = 0.08).

LITERATURE CITED

1. Wilson, P. W., *Bacteriol. Rev.*, **21**, 215–26 (1957)
2. Wilson, P. W., in *Handbook of Plant Physiology*, **8**, 9–47 (1958)
3. Allen, E. K., and Allen, O. N., in *Handbook of Plant Physiology*, **8**, 48–118 (1958)
4. Nutman, P. S., *Biol. Revs. Cambridge Phil. Soc.*, **31**, 109–51 (1956)
5. Shields, Lora M., *Botan. Rev.*, **19**, 321–76 (1953)
6. Jensen, H. L., *Bacteriol. Rev.*, **18**, 195–213 (1954)
7. Fogg, G. E., *Ann. Rev. Plant Physiol.*, **7**, 51–70 (1956)
8. Virtanen, A. I., *Proc. Intern. Congr. Biochem., 3rd Congr., Brussels, 1955*, 425–33 (1956)
9. Virtanen, A. I., and Rautanen, N., in *The Enzymes*, **2**, 1089–1130 (1952)
10. Nicholas, D. J. D., in *Nutrition of the Legumes*, 239–53 (Butterworths Scientific Publications, London, Eng., 359 pp., 1959)
11. Bond, G., in *Nutrition of the Legumes*, 216–31 (Butterworths Scientific Publications, London, Eng., 359 pp. 1958)
12. Bond, G., *Advance. of Sci.*, **60**, 382–86 (1959)
13. Leaf, G., *Advance. of Sci.*, **60**, 386–92 (1959)
14. Toivonen, N. J., and Tommila, E., Eds., *Biochemistry of Nitrogen* (Suomalainen Tiedakatemia, Helsinki, Finland, 535 pp., 1955)
15. McElroy, W. D., and Glass, B., Eds., *Inorganic Nitrogen Metabolism* (Johns Hopkins Press, Baltimore, Md., 728 pp., 1956)

16. Hallsworth, E. G., Ed., *Nutrition of the Legumes* (Butterworths Scientific Publications, London, Eng., 359 pp., 1958)
17. Jensen, V., *Arch. Mikrobiol.*, **29**, 348–53 (1958)
18. Proctor, M. H., and Wilson, P. W., *Nature*, **182**, 891 (1958)
19. Hino, S., and Wilson, P. W., *J. Bacteriol.*, **75**, 403–408 (1958)
20. Roy A. B., and Mukherjee, M. K., *Nature*, **180**, 236 (1957)
21. Metcalfe, G., and Brown, M. E., *J. Gen. Microbiol.*, **17**, 567–72 (1957)
22. Brown, M. E., and Metcalfe, G., *Nature*, **180**, 282 (1957)
23. Nemeth, G., *Nature*, **183**, 1460–61 (1959)
24. Stephenson, G., *Nature*, **182**, 1523–24 (1958)
25. Delwiche, C. C., and Wijler, J., *Plant and Soil*, **7**, 113–29 (1956)
26. Dugdale, R., Dugdale, V., Neess, J. and Goering, J., *Science*, **130**, 859–60 (1959)
27. Alexander, M., and Wilson, P. W., *Appl. Microbiol.*, **2**, 135–40 (1953)
28. Hutner, S. H., Provasoli, L., Schatz, A., and Haskins, C. P., *Proc. Am. Phil. Soc.*, **94**, 152–70 (1950)
29. Allen, M. B., and Arnon, D. I., *Plant Physiol.*, **30**, 366–72 (1955)
30. Evans, H. J., *Soil Science*, **81**, 199–208 (1956)
31. Nicholas, D. J. D., *Ann. Botany (London)*, **21**, 587–98 (1957)
32. Hewitt, E. J., in *Nutrition of the Legumes*, 15–41 (Butterworths Scientific Publications, London, Eng., 359 pp., 1958)
33. Nason, A., in *Trace Elements*, 269–96 (Academic Press, New York, N.Y., 410 pp., 1958)
34. Esposito, R. G., *Trace Metals in the Nutrition of Azotobacter vinelandii O.* (Master's thesis, Univ. of Wisconsin, Madison, Wis., 1954)
35. Esposito, R. G., and Wilson, P. W., *Proc. Soc. Exptl. Biol. Med.*, **93**, 564–67 (1956)
36. Mulder, E. G., *Plant and Soil*, **1**, 94–119 (1948)
37. Takahashi, H., and Nason, A., *Biochim. et Biophys. Acta.*, **23**, 433–35 (1957)
38. Keeler, R. F., and Varner, J. E., in *Trace Elements*, 297–309 (Academic Press, New York, N.Y., 410 pp., 1958)
39. Davies, E. B., and Stockdill, S. M. J., *Nature*, **178**, 866 (1956)
40. Norris, J. R., and Jensen, H. L., *Nature*, **180**, 1493–94 (1957)
41. Eyster, C., *Nature*, **170**, 755 (1952)
42. Gerretsen, F. C., and deHoop, H., *Plant and Soil*, **5**, 349–67 (1954)
43. Holm-Hansen, O., Gerloff, C. G., and Skoog, F., *Physiol. Plantarum*, **7**, 665–75 (1954)
44. Levin, A. P., Funk, H. B., and Tendler, M. D., *Science*, **120**, 784 (1954)
45. Virtanen, A. I., and Lundbom, S., *Acta Chem. Scand.*, **8**, 870 (1954)
46. Carnahan, J. E., and Castle, J. E., *J. Bacteriol.*, **75**, 121–24 (1958)
47. Bornstein, B. T., and Barker, H. A., *J. Bacteriol.*, **55**, 223–30 (1948)
48. Anderson, A. J., in *Inorganic Nitrogen Metabolism*, 3–58 (The Johns Hopkins Press, Baltimore, Md., 728 pp., 1956)
49. Raggio, M., Raggio, N., and Torrey, J. G., *Am. J. Botany*, **44**, 325–34 (1957)
50. Bergersen, F. J., *J. Gen. Microbiol.*, **19**, 312–23 (1958)
51. Mozen, M. M., Burris, R. H., Lundbom, S., and Virtanen, A. I., *Acta Chem. Scand.*, **9**, 1232 (1955)
52. Hiai, S., Mori, T., Hino, S., and Mori, T., *J. Biochem. (Tokyo)*, **44**, 839–47 (1957)
53. Hino, S., *J. Biochem. (Tokyo)*, **42**, 775–84 (1955)
54. Pratt, D. C., and Frenkel, A. W., *Plant Physiol.*, **34**, 333–37 (1959)

55. Pengra, R. M., and Wilson, P. W., *J. Bacteriol.*, **75,** 21–25 (1958)
56. Rosenblum, E. D., and Wilson, P. W., *J. Bacteriol.*, **59,** 83–91 (1950)
57. Burris, R. H., Magee, W. E., and Bach, M. K., in *Biochemistry of Nitrogen,* 190–99 (Suomalainen Tiedeakatemia, Helsinki, Finland, 535 pp., 1955)
58. Parker, C. A., and Scutt, P. B., *Biochim et Biophys. Acta,* **29,** 662 (1958)
59. Wilson, T. G. G., and Roberts, E. R., *Biochim. et Biophys. Acta,* **15,** 390–94 (1954)
60. Taniguchi, S., Mitsui, H., Nakamura, K., and Egami, F., in *Biochemistry of Nitrogen,* 200–15 (Suomalainen Tiedakatemia, Helsinki, Finland, 535 pp., 1955)
61. Hofman, T., and Lees, H., *Biochem. J.*, **54,** 579–83 (1953)
62. Suzuki, N., and Suzuki, S., *Sci. Repts., Tohoku Univ., Fourth Ser.,* **20,** 195–201 (1954)
63. Spencer, D., Takahashi, H., and Nason, A., *J. Bacteriol.,* **73,** 553–62 (1957)
64. Saris, N., and Virtanen, A. I., *Acta Chem. Scand.,* **11,** 1438–40 (1957)
65. Rautanen, N., in *Handbook of Plant Physiology,* **8,** 212–23 (1958)
66. Bach, M. K., *Biochim. et Biophys. Acta,* **26,** 104–113 (1957)
67. Bach, M. K., and Burris, R. H., *Federation Proc.,* **16,** 148 (1957)
68. Bulen, W. A., and Doughty, C. C., *Plant Physiol.,* **33,** Suppl. xi (1958)
69. Burris, R. H., in *Inorganic Nitrogen Metabolism,* 316–43 (Johns Hopkins Press, Baltimore, Md., 727 pp., 1956)
70. Allison, R. M., and Burris, R. H., *J. Biol. Chem.,* **224,** 351–64 (1957)
71. Wilson, P. W., *Advances in Enzymol.,* **13,** 345–75 (1952)
72. Thompson, J. F., Morris, C. J., and Gering, R. K., *Anal. Chem.,* **31,** 1028–30 (1959)
73. Krebs, H. A., *Biochem. J.,* **43,** 51–57 (1948)
74. Leaf, G., Gardner, I. C., and Bond, G., *J. Exptl. Botany,* **9,** 320–31 (1958)
75. Keilin, D., and Wang, Y. L., *Nature,* **155,** 227–29 (1945)
76. Smith, J. D., *Biochem. J.,* **44,** 591–98 (1949)
77. Keilin, D., and Smith, J. D., *Nature,* **159,** 692–94 (1947)
78. Virtanen, A. I., Jorma, J., Linkola, H., and Linnasalmi, A., *Acta Chem. Scand.,* **1,** 90–111 (1947)
79. Hamilton, P. B., Shug, A. L., and Wilson, P. W., *Proc. Natl. Acad. Sci., U.S.,* **43,** 297–304 (1957)
80. Thorogood, E., *Science,* **126,** 1011–12 (1957)
81. Bonner, W. D., Jr., *Ann. Rev. Plant Physiol.,* **8,** 427–52 (1957)
82. Yocum, C. S., and Hackett, D. P., *Plant Physiol.,* **32,** 186–91 (1957)
83. Cheniae, G., and Evans, H. J., *Biochim. et Biophys. Acta,* **26,** 654–55 (1957)
84. Elliot, W. H., *J. Biol. Chem.,* **201,** 661–72 (1953)
85. Weimberg, R., and Duodoroff, M., *J. Biol. Chem.,* **217,** 607–24 (1955)
86. Hoch, G. E., and Westlake, D. W., *Federation Proc.,* **17,** 243 (1958)
87. Wilson, P. W., and Burris, R. H., *Bacteriol. Rev.,* **11,** 41–73 (1947)
88. Bayliss, N. S., *Australian J. Biol. Sci.,* **9,** 364–70 (1956)
89. Hoch, G. E., Little, H. N., and Burris, R. H., *Nature,* **179,** 431–32 (1957)
90. Gest, H., in *Phosphorus Metabolism,* **2,** 522–43 (Johns Hopkins Press, Baltimore, Md., 930 pp., 1952)
91. Packer, L., and Vishniac, W., *Biochim. et Biophys. Acta,* **17,** 153 (1955)
92. Koepsell, H. J., Johnson, M. J., and Meek, J. S., *J. Biol. Chem.,* **154,** 535–47 (1944)
93. Winfield, M. E., *Revs. Pure and Appl. Chem. (Australia),* **5,** 217–46 (1955)

ENERGY TRANSFER THROUGH PHOSPHORYLATION MECHANISMS IN PHOTOSYNTHESIS[1,2]

By Otto Kandler

Bacteriological Institute of the Southern German Dairy Research and Experimental Station, Weihenstephan, Post Freising/Germany

In the first review of "phosphorous metabolism and photosynthesis" in this journal, the history of the problem was recorded by Arnon (3) in such an excellent way, that it seems superfluous to mention it again here.

By the end of 1955, there was essential experimental support for the hypothesis of Ruben (37), which stated that energy-rich phosphate formed with the help of light energy can be used for the reduction of CO_2. Different experiments had shown a clear effect of a light-dark change on the phosphorous metabolism of whole cells [Kandler (28); Wassink & Rombach (47); Simonis & Grube (40)]. Moreover it had been demonstrated that isolated chloroplasts can transform P_i (inorganic phosphate) into ATP in the light [Arnon *et al.* (5)]. It is intended in the present paper to continue Arnon's review by discussing the facts and hypotheses on the mechanism and importance of phosphorylation processes in photosynthesis.

The Cofactors of Photosynthetic Phosphorylation

Redox substances.—In the first experiments to demonstrate directly the transformation of light energy into energy-rich phosphate bonds, FMN (flavin mononucleotide) and ascorbate were used as cofactors for the chloroplast preparations [Whatley *et al.* (51)]. Later [Arnon *et al.* (6); Wessels (48, 49)] vitamin K_5, menadione (vitamin K_3), and phthiocol were also successfully applied.

In addition to these redox substances which occur naturally in the cell, Jagendorf & Avron (26) used various synthetic dyes, which also yielded very good rates of phosphorylation (Table I). With phenazine methosulfate (PMS) the phosphorylation rates were as much as five times higher than those found with the natural catalysts FMN or vitamin K. Therefore, the authors pointed out that the effectiveness of a compound *in vitro* gives no indication of its participation in photosynthetic phosphorylation *in vivo*.

[1] The survey of literature pertaining to this article was concluded in June 1959.

[2] The following abbreviations will be used: AMP (adenosine monophosphate); CMU (chlorophenyldimethyl urea); FMN (flavin mononucleotide); ~P (high-energy phosphate); PCR (photosynthetic carboxyl reductase); PGA (phosphoglyceric acid); P_i (inorganic phosphate); PMS (phenazine methosulfate); PN (pyridine nucleotide); PNH (pyridine nucleotide, reduced form); PPNR (photosynthetic pyridine nucleotide reductase); RuDP (ribulose diphosphate); TPNH (triphosphopyridine nucleotide, reduced form).

TABLE I

SURVEY OF THE ADDENDA AND RATES OF PHOTOSYNTHETIC PHOSPHORYLATION

Author	Addenda	Redox or Hill substance	End product	μM P_i esterified/hr./mg. chlorophyll
Whatley *et al.* (51)	Whole chloroplasts Tris buffer pH 7.2 KCl, AMP, P_i, $MgCl_2$.	FMN, (Ascorbate)	(ATP)	26.0
Arnon *et al.* (6)	Whole chloroplasts Tris buffer pH 7.2 KCl, AMP, P_i, $MgCl_2$.	Vitamin K_5 Menadione Phthiocol	(ATP)	?
Jagendorf & Avron (26)	Whole chloroplasts Tris buffer pH 7.8 P_i, ADP, NaCl, $MgCl_2$.	FMN Phenzine methosulfate Menadione *p*-Phenylendiamine Indigo carmine Methyl viologene Benzyl viologene	(ATP)	158–123 555–735 136–202 15– 31 159–175 110–156 92–153
Allen *et al.* (1)	Chloroplast fragments Chloroplast extract Tris buffer pH 8.3 P_i, ADP, $MgCl_2$ Ascorbate.	FMN+Vitamin K_5	(ATP)	360–519
Arnon *et al.* (8)	Broken chloroplasts Tris buffer pH 8.3 P_i, $MgCl_2$, ADP Chloroplast extract	TPN Ferricyanide	TPNH, O_2, ATP Ferrocyanide, O_2, ATP	72.0 (calculated from the curve) 130.0 (calculated from the curve)
Avron & Jagendorf (10)	Whole chloroplasts Tris buffer pH 7.8 P_i, NaCl, ADP, $MgCl_2$ PPNR.	TPN Ferricyanide	TPNH, O_2, ATP Ferrocyanide O_2, ATP	91.0 83.0

Even though the strong increase in the rate of phosphorylation caused by PMS is especially striking, the effectiveness of methyl viologene and benzyl viologene appears to be theoretically more important. In contrast to all redox systems applied so far, both show a very negative redox potential, namely −0.46 v. and −0.32 v.

As opposed to the Hill reaction in which oxygen is evolved and the added redox substance is accumulated in the reduced form, no oxygen is given off and the cofactors mentioned above presumably act as catalysts in photosynthetic phosphorylation. The only net change of the system is the transformation of P_i + ADP into ATP as in reaction 1.

$$P_i + ADP + \text{light} \xrightarrow[Mg^{++},\ FMN,\ etc.]{\text{chloroplasts}} ATP \qquad 1.$$

In 1958 Arnon *et al.* (8) made a very important discovery. By adding chloroplast extracts to broken chloroplasts (broken chloroplasts were obtained by suspending whole chloroplasts in water, i.e., hypotonic solution) the following reactions (2 and 3) could be demonstrated:

$$TPN + ADP + P_i + \text{light} \xrightarrow[Mg^{++},\ \text{chloroplast extract}]{\text{broken chloroplast}} TPNH + ATP + \tfrac{1}{2}\ O_2 \qquad 2.$$

$$2 \text{ ferricyanide} + ADP + P_i + \text{light} \xrightarrow[Mg^{++}]{\text{broken chloroplasts}} 2 \text{ ferrocyanide} + 1\ ATP + \tfrac{1}{2}\ O_2 \qquad 3.$$

Both reactions, which have been confirmed qualitatively and quantitatively by Jagendorf (25), reveal a series of important facts:

(*a*) Despite its very negative redox potential (−0.32 v.), TPN can function as a Hill reagent. Previously, the reduction of TPN by illuminated chloroplasts had been clearly demonstrated by San Pietro & Lang (39), but it had not been possible to show a simultaneous release of oxygen or stoichiometric phosphorylation.

(*b*) The reduction of TPN is dependent on the participation of a specific factor, which can easily be eluted from chloroplasts. San Pietro (38) was able to purify partly and characterize this enzyme, which he called photosynthetic pyridine nucleotide reductase (PPNR).

(*c*) TPNH and ATP are formed simultaneously and are not products of alternative reactions.

(*d*) The classical Hill reaction with ferricyanide can also be coupled with phosphorylation. Indeed, the rate of O_2 evolved is greater when the reaction is coupled with phosphorylation by the addition of ADP. After the discovery of the TPN-coupled phosphorylation, the term cyclic phosphorylation, describing the synthesis of ATP without O_2-evolution mediated by FMN, vitamin K, etc., was substituted by Arnon *et al.* (8) for the

older term photosynthetic phosphorylation. They designate the formation of ATP with oxygen evolution and TPN reduction the "one-step" or direct phosphorylation. Thus, the general term photosynthetic phosphorylation is now subdivided into cyclic and direct phosphorylation.

Phosphate acceptor.—AMP (adenosine monophosphate) was used as a phosphate acceptor in the early experiments. Later, however, in experiments with chloroplasts [Allen *et al.* (1)] as well as with chromatophores of purple bacteria [Frenkel (20)], it was found that ADP is the actual phosphate acceptor. In crude preparations where AMP is also effective as phosphate acceptor, ADP is regenerated by myokinase from AMP and ATP.

Ions.—Among the metal ions, Mg^{++}, which can be replaced by Co^{++} but not by Mn^{++}, proved to be essential [Arnon (4), Mudd (35)]. Latzko & Mechsner (32) and Mechsner (34) were able to show that K^+ influences photosynthetic phosphorylation in whole cells and that Rb^+ can be substituted for K^+.

Stabilizing agents.—Originally ascorbic acid [Arnon (2)] was regarded to be important as a redox catalyst, like FMN, etc. According to recent investigations by Wessels (48) and Arnon *et al.* (7) ascorbate is a stabilizing factor rather than a redox catalyst. This point of view was supported by Füchtbauer & Simonis (22) who were able to show that ascorbate is required only in chloroplasts of young leaves.

Hypotheses on the Mechanism of Photosynthetic Phosphorylation

So far, all the hypotheses on the mechanism of light phosphorylation are based on the assumption that the reductant [H], generated by light absorption in the chlorophyll, is more or less completely reoxidized. This reoxidation is believed to proceed along a chain of redox catalysts via several steps. At least one, if not all, of these steps to more positive redox potentials, is thought to be coupled with the formation of ~P (high-energy phosphate), analogous to oxidative phosphorylation.

As early as 1942 this concept was expressed by Ruben (37) and later by Kok (31). In 1950 Kandler (28) discussed it more fully by suggesting two alternative schemes.

Scheme I (Fig. 1) is based on the assumption that the energy of one light quantum (1 Einstein quantum of red light equals 43 kcal.) raises

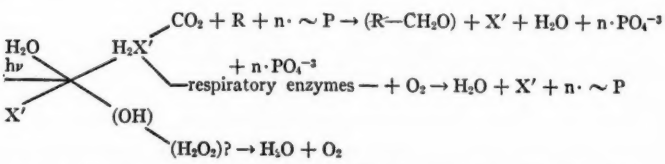

$CO_2 + R + n\cdot \sim P \rightarrow (R{=}CH_2O) + X' + H_2O + n\cdot PO_4^{-3}$
H_2O
$h\nu$
H_2X'
$+ n\cdot PO_4^{-3}$
respiratory enzymes $- + O_2 \rightarrow H_2O + X' + n\cdot \sim P$
X'
(OH)
$(H_2O_2)? \rightarrow H_2O + O_2$

Fig. 1. Scheme I of Photosynthetic Phosphorylation [Kandler (28)], $X'/X'H_2$ has a Redox Potential Like a Flavine.

one electron pair from the system H_2O/O_2 ($E_0 = 0.81$ v.) to a redox system of about 0.0 v., e.g., flavin enzyme (0.1 v potential difference/electron equivalent equals 2 kcal.ΔF.). One part of the reduced flavin reacts with O_2, in a system similar to the respiration chain forming ATP, while the other part in which ATP is consumed is used for the reduction of CO_2.

Once the catalytic effect of FMN and other redox catalysts with an E_0 between 0.0 v. and -0.2 v. on the photosynthetic phosphorylation of isolated chloroplasts was detected, similar, more or less modified schemes were fre-

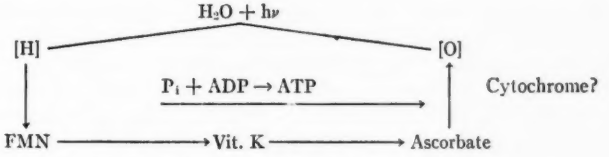


FIG. 2. Early Diagram of Photosynthetic Phosphorylation Modified after Arnon (6) and Wessels (48).

quently proposed. Arnon *et al.* (6) and Wessels (48) assumed that the scheme shown in Figure 2 is realized in the chloroplasts. Because photosynthetic phosphorylation in whole *Chlorella* cells [Kandler (29)], in purple bacteria [Frenkel (19)], and in chloroplasts [Whatley *et al.* (51)] requires no molecular oxygen, a recombination with the oxidant formed in the light is suggested in this scheme. None of the authors have specified at which of the numerous steps ~P is generated.

Scheme II by Kandler (Fig. 3) is based on the assumption that not a

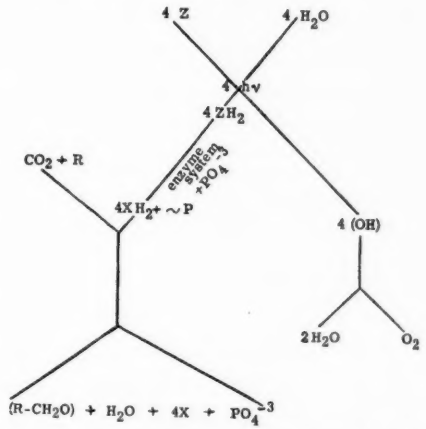


FIG. 3. Scheme II of Photosynthetic Phosphorylation [Kandler (28)], X/XH_2 Has a Redox Potential Like TPN/TPNH.

pair of electrons but a single electron is activated by absorption of the energy of one quantum. Consequently, it could be transferred to a redox system with an E_0 of about -1.0 v. Although one has to assume that there is a considerable loss in energy during the electron transfer from the different activated stages of chlorophyll to the first defined redox substance [see also Duysens (17)], there is still sufficient energy to reach a redox potential considerably more negative than that of the PN (-0.32 v.). This hypothetical first redox system, with a very negative potential, is designated as Z/ZH in Scheme II. With the help of one or more enzymes, [H] would be transferred to a redox system X/XH, with a potential similar to PN (pyridine nucleotide). This electron transfer is thought to be coupled with phosphorylation, and subsequently, CO_2 could be reduced with the help of this newly formed ~P. For this concept it is necessary to assume that the catalyst Z/ZH is able to collect two electrons and can then transfer them together. Barltrop *et al.* (11) have discussed mechanisms of this kind in more detail.

By demonstrating the simultaneous formation of ATP and TPNH in chloroplasts, Arnon *et al.* (8) after eight years provided the first experimental evidence for this purely hypothetical scheme. They consider CO_2 to be reduced by ATP and TPNH and thus call these two cofactors the "assimilatory power." When CO_2 is limiting, the hydrogen is thought to be transferred to FMN by diaphorase and then to [O], according to the above mentioned scheme for cyclic phosphoylation (Fig. 2). Eventually all the utilizable light energy occurs in the form of ATP.

To demonstrate the combination of the "one step" phosphorylation with cyclic phosphorylation in chloroplasts, Arnon *et al.* (8) included FMN or vitamin K in addition to TPN. The TPN-induced O_2 evolution was completely blocked by the two cofactors of cyclic phosphorylation and the rate of phosphorylation was considerably increased. In other experiments a definite amount of TPN was combined with varying amounts of FMN [Arnon (4)]. The TPN-dependent increase in the rate of phosphorylation was highest with suboptimal amounts of FMN, and decreased with increasing amounts of FMN. Arnon (4) concluded from these experiments that the FMN addition changes the "one-step" phosphorylation to cyclic phosphorylation, as shown in the scheme presented in Figure 4.

Although a phosphorylation supplied with energy by the electron trans-

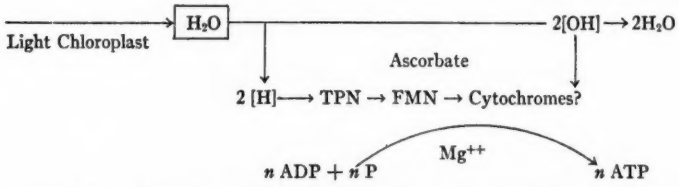


FIG. 4. Diagram Representing Cyclic Photophosphorylation by the FMN Pathway [Arnon (4)].

fer from [H] to TPN, is a very tempting idea, other possible explanations must not be overlooked. In fact, Bassham (12) considers it possible that ATP is formed not by an electron transfer to TPN or ferricyanide, but by the formation of O_2 and H_2O from the oxidant as shown in reaction 4.

$$2\ [OH] + ADP + P_i \xrightarrow{Mn^{++}} \tfrac{1}{2}\ O_2 + ATP + H_2O \qquad 4.$$

Arnon (4) rejected this assumption and points to the fact that no oxygen is evolved in cyclic phosphorylation. This argument is not satisfactory however, for we do not know if both kinds of light-induced phosphorylation, cyclic and "one-step," proceed by the same reaction mechanism. Moreover, it is not certain if the energy for ATP-synthesis is supplied by an electron transfer from [H] to FMN in cyclic phosphorylation. ATP-formation could also be coupled with the oxidation of FMN by the oxidant. This latter proposal is analogous to photosynthetic phosphorylation in purple bacteria, where the formation of ATP is probably coupled to the oxidation of cytochrome-c_2 by the oxidant [Frenkel (21)].

Avron & Jagendorf (10) carried out some very important experiments to elucidate the reaction mechanisms of photosynthetic phosphorylation. The results of those experiments, intended to demonstrate a multi-step phosphorylation by combination of TPN and FMN, are contained in Table II.

TABLE II

INDEPENDENCE OF CYCLIC PHOSPHORYLATION WITH RIBOFLAVINE PHOSPHATE FROM PHOTOSYNTHETIC PYRIDINE NUCLEOTIDE REDUCTASE

All values shown are in μM/mg. of chlorophyll/h. The reaction time was 5 min. Reaction mixtures contained 10 μM each of phosphate and ADP, and 5×10^4 c.p.m. of P^{32}. ATP formation was measured by absorption of ATP on charcoal, then subsequent hydrolysis of the radioactive phosphate with 1.0 *N* HCl [Avron & Jagendorf (10)].

Cofactor or oxidant	Additions	Plastids washed once		Plastids washed 5 times	
		TPN reduced	ATP formed	TPN reduced	ATP formed
TPN, 1.5 μM		0	11	0	6
	PPNR*	125	91	97	61
Riboflavin phosphate, 0.2 μM			122		80
	TPN, 0.2 μM		120		72
	TPN+PPNR		127		88
	TPN+PPNR+TPNH diaphorase		122		78

* Photosynthetic pyridine nucleotide reductase.

Evidently, in the five times washed chloroplasts, the phosphorylation rate is increased by PPNR only if TPN and not riboflavin-phosphate is used as a cofactor. The combination of riboflavin-phosphate plus TPN does not respond to PPNR. Since the combination of both cofactors does not increase the phosphorylation rate beyond that achieved by the most effective partner alone, Avron & Jagendorf (10) suggest that the reduction of TPN is sidestepped in the presence of riboflavin-phosphate. In Arnon's experiments mentioned above, suboptimal concentrations of FMN were always used and therefore his results and those of Avron & Jagendorf are difficult to compare. It would be interesting to know if in an experiment with low FMN concentrations the TPN-stimulation of phosphorylation is PPNR-dependent. The results from such an experiment would show clearly whether FMN is reduced either by TPNH [Arnon (4)], or directly by [H] [Avron & Jagendorf (10)]. It is likely that both hypotheses are correct, since at low concentrations FMN may have a synergistic effect, in the sense of Arnon, while at high concentrations it could compete directly with TPN for the oxidation of [H].

Further experiments by Avron & Jagendorf (10) elucidate the correlation between the Hill reagents and the cofactors of the cyclic phosphorylation. When 2,3,6-trichlorophenol-indophenol dye, which allows O_2 evolution but no phosphorylation, was combined with PMS, phosphorylation was interrupted until all the dye was reduced. One might expect *p*-chlorophenyldimethyl urea (CMU), which inhibits the Hill reaction and not the cyclic phosphorylation, to remove the inhibition of cyclic phosphorylation caused by the Hill reagents. Instead, because of the inhibition of the Hill reaction, CMU prolongs the inhibition to a degree corresponding to the delayed reduction of the dye. Since the oxidized form of the dye itself acts as an inhibitor, the inhibition of the cyclic phosphorylation by the Hill reagents cannot be explained simply by the trapping of electrons. The same is true for the combination of ferricyanide plus PMS, where the phosphorylation rate is also inhibited as long as ferricyanide is present. Only after its reduction, does the much higher rate of phosphorylation caused by PMS start. The quick disappearance of the inhibition after the reduction of the Hill oxidants suggests that they "inhibit by virtue of a firm binding to the enzymatic site(s) at which they are normally reduced" (10).

Avron & Jagendorf (10) come to the conclusion that each Hill reagent and each cofactor of cyclic phosphorylation "starts out with the same early intermediates from the cleavaging of water," as summarized in the scheme shown in Figure 5. According to this scheme ATP would be formed by a one-electron transfer, the site of which is not exactly specified.

Avron & Jagendorf (9) also performed experiments to determine whether P_i or ADP was the first to react with an intermediate of light phosphorylation. The two most important results are: (*a*) Arsenate, which can replace P_i but not ADP, inhibits the formation of ATP from ADP in a Hill reaction activated by the addition of P_i and ADP. (*b*) When

chloroplasts are incubated with $P^{32}{}_i$ and ATP, or with ATP^{32} and P_i, there is no exchange of activity between the two compounds. Also, arsenate does not bring about any exchange.

If the reaction were analogous to oxidative phosphorylation, where a strong exchange from $P_i \longleftrightarrow ATP$ as well as from $O_2 \longleftrightarrow H_2O$ takes place, these results would not be expected. Therefore, Avron & Jagendorf

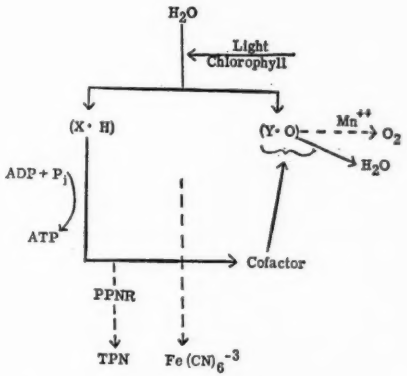


FIG. 5. Minimal Scheme for Interaction of Cofactors and Oxidants with Chloroplast Electron Transport Pathway [Avron & Jagendorf (10)].

(9) conclude that in contrast to oxidative phosphorylation, the last step of photosynthetic phosphorylation is irreversible and ADP, not P_i, is bound to an intermediate of the phosphorylation reaction. Avron & Jagendorf suggest the following sequence of reactions:

(*a*) $H_2O + X + Y \longrightarrow XH + YO$

(*b*) $YO \longrightarrow Y + O_2$

(*c*) $XH + J + A \longrightarrow X + J^x + A\ H$

(*d*) $AH + Fe(CN)_6^{-3} \longrightarrow A + H^+ + Fe(CN)_6^{-4}$

(*e*) $J^x + ADP \longrightarrow J \sim ADP$

(*f*) $J \sim ADP + P_i \longrightarrow J + ATP$

XH and YO stand for the first products of photolysis. AH is the compound that reduces ferricyanide. During the electron transfer from XH to AH, compound J, necessary for the electron transfer is activated to J^x. It can only be regenerated by the formation of an adenylate and subsequent phosphoroclastic cleavage.

In Figure 6 the presently known cofactors of cyclic phosphorylation and the most important Hill reagents are compiled. In the diagram the cofactors are plotted along a redox potential scale. The direction of the arrows

indicates which of the two processes is working when the cofactor in question is used.

It is interesting to see that all substances with a positive redox potential serve as Hill reagents, and those with a negative potential as catalysts in the cyclic phosphorylation. Ascorbate, which may not act as a catalyst as originally assumed, would be the only exception. Ascorbate was placed in brackets in the scheme since, as mentioned earlier, it is now regarded as a stabilizer.

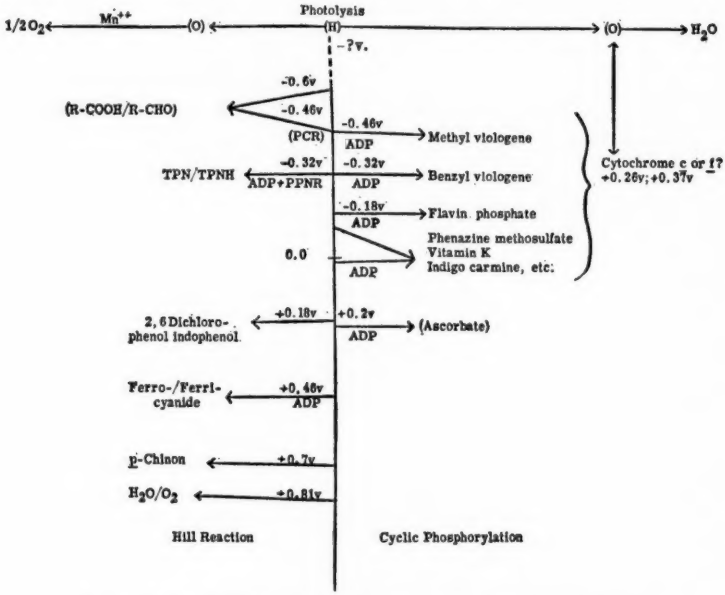


FIG. 6. Redox Potential Scale with the Cofactors of Hill Reaction and Cyclic Phosphorylation.

TPN with a very negative redox potential is an exception on the Hill reaction side of the scheme. In contrast to all the other Hill reagents, a specific enzyme PPNR is needed for its reduction. Thus the TPN-reduction which is comparable to the diaphorase reaction, represents a type of reaction *sui generis* among the Hill reactions.

If by the term Hill reagent one includes all compounds that cause a stoichiometric release of O_2 during their reduction by illuminated chloroplasts, CO_2 must be included in this group as well. The redox potential of the system R-COOH/R-CHO depends on the nature of R and is about —0.55 v. for a 3-carbon compound or —0.46 v. for a 6-carbon compound.

Since even compounds as negative as TPN and benzyl- or methyl viologene can be reduced while ATP is formed, the redox potential of the original reductant has to be more negative by at least 0.2 v., i.e., less than −0.6 v. Thus, from an energetic point of view, a direct reduction of the carboxyl group is possible without the assistance of ATP. By analogy to the enzymatic reduction of TPN, one has only to postulate a "photosynthetic carboxyl reductase" (PCR) and the system R-ROOH/R-CHO fits readily in the scheme. Many biochemists who have definitely accepted Calvin's photosynthetic cycle, will think this suggestion superfluous, but one must keep an open mind.

Unfortunately, thus far nothing is known of the redox potential or the chemical form of the oxidant. There is some good reason for the assumption that it has a more negative redox potential than that of H_2O/O_2. Spectrophotometric measurements during the light-dark change indicate that cytochromes are involved in the oxidative side of photosynthesis [Kamen (27), Vernon (45), Wessels (49), and Duysens (16)]. It is possible that compounds with a positive redox potential trap the electrons and thus prevent a recombination of [H] and [O], whereas all the compounds with a negative potential function as catalysts and can be reoxidized by the [O] via cytochromes.

If one assumes that light-induced phosphorylation occurs in a single and unique reaction, independent of a specific cofactor, then the adenylate suggested by Avron & Jagendorf would have to be formed simultaneously with the direct oxidation of [H] by any one of the mentioned catalysts. Theoretically there are sufficient possibilities to form an electron ladder for further phosphorylation steps, analogous to oxidative phosphorylation as suggested by Arnon. It is not absolutely certain, however, whether or not these opportunities are utilized by the chloroplasts.

Alternative hypotheses, stating that ATP is formed either by the formation of O_2 from the [O], or by the oxidation rather than the reduction of the cofactors of the cyclic phosphorylation, cannot be excluded on the basis of the known facts.

THE IMPORTANCE OF LIGHT PHOSPHORYLATION FOR THE PHOTOSYNTHETIC CO_2 ASSIMILATION

Assumptions

The very well known scheme of Calvin and coworkers [see Calvin & Bassham (15)] expresses clearly the necessity of ATP for the photosynthetic CO_2-assimilation. According to this scheme three moles of ATP are required for the reduction of one mole of CO_2. Of these, two moles are needed for the reduction of PGA (phosphoglyceric acid) to triose phosphate, and one mole is required for the phosphorylation of ribulose-5-phosphate to RuDP (ribulose diphosphate).

Other hypotheses, which are less well supported by experimental evi-

dence, state that much less ATP is necessary for CO_2-assimilation. Frank (18) for example, considers that a direct photochemical reduction of PGA, requiring neither a specific redox system nor ATP is possible. Thus, only one mole of ATP required for the regeneration of the CO_2-acceptor, RuDP, would be necessary for the reduction of one mole of CO_2.

Warburg & Krippahl (46) hold a similar point of view since they believe in a direct photo-dissociation of carbon dioxide by light without the interference of ATP. They give no details concerning either the participation of ATP in the reactions following the reduction of CO_2 or the possible formation of ATP from light energy.

Kandler (30) proposed recently that the carboxylation product of RuDP, which according to Calvin is a β-keto acid, could be directly reduced to the carbohydrate level. He suggests, in fact, that the main portion of radioactivity found in PGA after short-time photosynthesis in $C^{14}O_2$, originates from a non-enzymatic splitting of the β-keto acid during the extraction of the cells. In addition, if there is not enough reducing power *in vivo*, a part of the β-keto acid could be split to PGA, in which case PGA would be transformed to amino acids and other compounds derived from pyruvate.

The redox system which reduces the β-keto acid is assumed to have a more negative potential than TPN/TPNH. Thus ATP would be required for the regeneration of the CO_2-acceptor and not for the reduction step. If fructose diphosphate and sedoheptulose diphosphate were not dephosphorylated by a phosphatase, but instead the ester phosphates were transferred to ribulose-5-phosphate, much less ATP would be required. In this case only the phosphate bonds used in the formation of polysaccharides would have to be continuously replaced, and a minimal ratio of 1 mole ATP/6 moles CO_2 would result. Of course actual demand for ATP would always be greater because organic phosphate is withdrawn from the cycle when intermediates are required for the synthesis of proteins and fats, etc., and also because some is lost by hydrolysis during the ester transfer. According to this view, large amounts of ATP are needed only during the induction phase of photosynthesis when the sugar-phosphate pools, which are much larger in the light, are built up. In fact, there would be no strong stoichiometry between ATP consumption and photosynthetic CO_2-assimilation.

Experiments

Many experiments with whole cells as well as with chloroplasts have been carried out in attempts to demonstrate directly the participation of ATP in CO_2-assimilation.

Experiments with whole cells.—According to the Calvin cycle, the overall change, as seen in reaction 5

$$\text{triose phosphate} + P_i + ADP + PN \leftrightarrow PGA + ATP + PNH \qquad 5.$$

goes from the left to the right in the dark and is reversed during photosynthesis. Bassham *et al.* (13) found, however, that the triose phosphate

concentration is higher and the PGA concentration is lower in light, than in darkness. Therefore, high concentrations of the cofactors ATP and PNH would have to be expected in the light in order to drive the reaction to the left against an unfavourable triose/PGA balance. Some authors have already tried to show such an increase in ATP concentration during photosynthesis.

Strehler (43), using the very sensitive fire-fly method, has detected only half as much ATP in the light as in the dark under aerobic conditions. When measuring the pool sizes in P^{32}-saturated cells, Goodman *et al.* (23) found 40 per cent less ATP in the light than in the dark. Moreover, Bradley's curves (14) for the amount of ATP obtained with P^{32} during the light-dark transition show a 10 per cent decrease in ATP with illumination in the presence of CO_2. Kandler (30) also observed a decrease rather than an increase of the 7-minute phosphate in the light. These experiments provide no evidence for the role of ATP as a force driving the reduction of CO_2 in whole cells.

Many publications deal with the determination of phosphate turnover during photosynthesis. If the phosphate turnover is calculated on the basis of the Calvin cycle where three moles of ATP are required for the reduction of one mole of CO_2, it should be 20 times higher in the light than in the dark with a fortyfold overcompensation of respiration. P^{32}-feeding provides the best method for investigating the increases in the phosphate turnover. The more frequently organic phosphate is transformed into inorganic phosphate in the cell per time unit, and vice versa, the better is the chance for the added tracer to be fixed in an organic binding. A twentyfold increase in the phosphate turnover ought therefore to yield a clear positive result.

The first experiments of this kind were performed by Goodman *et al.* (23), who investigated the distribution of radioactivity in *Scenedesmus* after incubating the cells with P^{32} for 37 sec. in the dark, and 29 sec. in the light. In darkness the ATP fraction contained 72 per cent of the total activity, and in the light only 9.8 per cent. This is interpreted by the authors as resulting from faster turnover in the light, leading to a faster distribution of P^{32} into fractions other than ATP. Correspondingly, in short-time experiments the ATP portion should increase considerably but unfortunately, there is as yet no experimental evidence for this.

In *Elodea,* Grube (24) observed a much higher total uptake of P^{32} in the light than in the dark, but this increase was lower by only 30 per cent when CO_2 was withdrawn. This indicates that the increased phosphate turnover depends only slightly on CO_2-assimilation. Simonis & Kating (41) who investigated the effect of light and glucose-feeding on the uptake of P^{32} by *Ankistrodesmus* found that both treatments resulted in a higher P^{32} uptake, and that light had an effect no greater than glucose-feeding. They chose a uniform incubation time of 5 min. but presented no data concerning the time needed for the saturation of the cell with P^{32}. It might well be that

their results represent the pool size after saturation and not the rate of saturation. Thus no statement on phosphate turnover is possible.

In more recent experiments on the distribution of P^{32}, Simonis & Weichart (42) failed to find an essential increase in the total fixation of P^{32} in the light by *Elodea* leaves. Indeed, the activities found in ADP and ATP were even slightly lower in the light than in the dark. Again, since no kinetic studies were performed, the relatively long experimental time of 3 min., followed by a 2 min. period for rinsing, hardly permit any statements regarding turnover.

Kandler (30), using the cyanide method which Lynen & Koenigsberger (33) had applied to yeast, attempted a direct determination of the rate of phosphorylation in *Chorella* under light and dark conditions. A P/O ratio of 2.8 for endogenous respiration and of 1.7 to 2.1 for glucose respiration was found in the dark. This agrees with the earlier results of Zöllner (52). Illumination caused a 50 per cent increase in the rate of phosphorylation without substrate, but in the presence of glucose no significant rise in the rate of phosphorylation was observed. Furthermore, the gas phase (with and without CO_2, N_2, air) had no influence on the rate of phosphorylation in the light. Consequently Kandler (30) comes to the conclusion that there is no stoichiometric ratio of 3:1 between light-induced phosphorylation and CO_2-assimilation.

In experiments with whole cells one must consider the possibility that large plasma pools could mask the changes occurring in the small chloroplast pools. If this were true, light should have no more than a very slight effect on the phosphate turnover. The above mentioned experiments show, however, that while light has a very pronounced effect on phosphate turnover, it unfortunately does not have the effect predicted by the Calvin scheme.

Experiments with chloroplasts.—Determination of the rate of phosphorylation is both more difficult and more ambiguous in whole cells than in chloroplasts. In the first publications on chloroplasts (Table I) low rates were reported, but recently rates up to 900 μM P_i esterified/mg. Chlorophyll/hr. have been found [Jagendorf & Avron (26)]. Allen *et al.* (1) point out that rates of phosphorylation such as this are sufficient to supply a maximal CO_2-assimilation (180 μM CO_2/mg. chlorophyll/hr.) with ATP in green leaves even when several moles of ATP are consumed per mole of CO_2 fixed. In this respect it is revealing to compare the rates of the electron flow in maximal cyclic phosphorylation with that of maximal CO_2-assimilation. One has to assume that one pair of electrons has to be transferred for the formation of one mole of ATP, while two pairs of electrons are necessary for the reduction of one mole of CO_2. Thus, 450 moles of electron pairs are recombined by cyclic phosphorylation at a phosphorylation rate of 900 μM P_i/hr./mg. chlorophyll. However, only about 360 moles of electron pairs are used for the reduction of CO_2 in whole cells at a maximal rate. Consequently, the chloroplasts are more efficient *in vitro* than *in vivo* as regards photolysis.

Regrettably, attempts to achieve in chloroplasts CO_2-assimilation rates like those in leaves, have been unsuccessful up to now. In general, the authors only give numbers for the fixed radioactivity, but no data which would permit the calculation of the molar amount of CO_2 fixed. Recently, however Arnon (4) has presented the quantitative data included in Table III on the CO_2-fixation of chloroplasts, in which the CO_2-fixation rate is only about 1 per cent of maximal photosynthesis. Here only 0.5 per cent of the reducing power [H], produced by the isolated chloroplasts, is utilized for CO_2-assimilation.

Trebst *et al.* (44) investigated the distribution of activity after $C^{14}O_2$-fixation in chloroplasts by paper chromatography. As in whole cells, most

TABLE III

CO_2 FIXATION BY ILLUMINATED CHLOROPLASTS FROM DIFFERENT PLANTS

The reaction mixture contained: Broken chloroplasts, chlorophyll-free extract, tris buffer pH 7.5, $MgCl_2$, $MnCl_2$, ascorbate, Na-phosphate, ADP, TPN, FMN, glucose-1-phosphate, $NaHC^{14}O_3$ [Arnon (4)].

Plant	CO_2 Fixed (μM/mg. chlorophyll/hr.)
Spinach	2.2
Sugar beet	3.5
Pokeweed	2.2
Tobacco	1.7
Sunflower	1.4
Tetragonia	2.0

of the activity was found in the phosphate esters, the mono- and diphosphate sugars, and PGA; but unlike whole cells, a much higher portion of this activity was found in triose phosphate.

When Trebst *et al.* (44) replaced illumination by the addition of ATP plus TPNH ("assimilatory power"), the phosphate esters were again the most active fraction, but in this case the portion of the diphosphates and of triosephosphate was lower and that of PGA higher. When the "assimilatory power" was produced either by a pre-illumination of the chloroplasts or by the addition of ATP and TPNH, similar results were obtained. The authors interpret their results as evidence for the absolute necessity of TPNH and APT for CO_2-reduction via PGA, which contained a big portion of the radioactivity on the chromatograms.

Previously Racker (36) had presented evidence showing that the synthesis of sugar from CO_2 is possible in the presence of a multi-enzyme system containing the carboxylating enzyme, the enzymes of glycolysis, and those of the pentose-phosphate cycle. The system of Racker, however, consists of enzymes from many sources in the animal and plant kingdoms,

whereas the system used by Trebst *et al.* (44) has the advantage of containing only natural substances of the plant cell. It is not certain, however, whether this allows one to speak of the latter system as a genuine model of photosynthetic CO_2-assimilation, and of Racker's as a test-tube artifact. Although the chloroplasts are very efficient in forming ATP and TPNH, only 1 per cent of the normal CO_2-fixation rate is realized and it is obvious that the system is not quite complete. It is easy to imagine that an important cofactor or enzyme for the genuine photosynthetic CO_2-reduction is missing. If this is so, the products of photolysis *quasi* have to form TPNH and ATP as an "ersatz assimilatory power" and the primary carboxylation product would be split to PGA. In the presence of triose phosphate-dehydrogenase, etc., PGA would be transformed to sugar phosphates by reversal of glycolysis, according to Racker's system. Of course, there is no sound evidence that this somewhat pessimistic explanation is correct, but still the reviewer cannot think of a fact which would exclude this interpretation.

Since the rate of phosphorylation obtainable from chloroplast preparations was raised by a factor of 50 within the last three years, one might hope that in a few years the rate of photosynthetic CO_2-fixation obtainable from isolated chloroplasts will also be increased to that of whole cells. The future will show whether, as in the case of photosynthetic phosphorylation, new cofactors and enzymes of photosynthetic CO_2-fixation are to be found as the rate is increased.

At present it appears to be too early to draw a definite conclusion on the importance of the energy transfer by phosphorylation processes in photosynthesis. Although it is certain that light energy can be very efficiently transferred in $\sim$P by recombination of the products of photolysis, it is not clear if and how much of this $\sim$P is used for the reduction of CO_2.

LITERATURE CITED

1. Allen, M. B., Whatley, F. R., and Arnon, D. I., *Biochim. et Biophys. Acta*, **27** 16–23 (1958)
2. Arnon, D. I., *Science*, **122**, 9–16 (1955)
3. Arnon, D. I., *Ann. Rev. Plant Physiol.*, **7**, 325–54 (1956)
4. Arnon, D. I., *Brookhaven Symposia in Biol.*, **11**, 181–233 (1958)
5. Arnon, D. I., Allen, M. B., and Whatley, F. R., *Nature*, **174**, 394–96 (1954)
6. Arnon, D. I., Whatley, F. R., and Allen, M. B., *Biochem. et Biophys. Acta*, **16**, 607–8 (1955)
7. Arnon, D. I., Whatley, F. R., and Allen, M. B., *Nature*, **180**, 182–85 (1957)
8. Arnon, D. I., Whatley, F. R., and Allen, M. B., *Science*, **27**, 1026–34 (1958)
9. Avron, M. and Jagendorf, A. T., *J. Biol. Chem.*, **234**, 967–72 (1959)
10. Avron, M. and Jagendorf, A. T., *J. Biol. Chem.*, **234**, 1315–20 (1959)
11. Barltrop, J. A., Hayes, P. M., and Calvin, M., *J. Am. Chem. Soc.*, **76**, 4348 (1954)
12. Bassham, J. A., *Brookhaven Symposia in Biol.*, **11**, 234 (1958)
13. Bassham, J. A., Shibata, K., Stenberg, K., Bourdon, J., and Calvin, M., *J. Am. Chem. Soc.*, **78**, 4120–24 (1956)
14. Bradley, D. F., *Arch. Biochem. Biophys.*, **68**, 172–85 (1957)
15. Calvin, M. and Bassham, J. A., *The Path of Carbon in Photosynthesis* (Prentice-Hall, Inc., New York 11, N.Y. 1957)
16. Duysens, L. N. M., *Science*, **121**, 210–11 (1955)
17. Duysens, L. N. M., *Ann. Rev. Plant Physiol.*, **7**, 25–50 (1956)
18. Frank, J., *Research in Photosynthesis*, 142–44 (Interscience Publishers, New York, N.Y., and London, England, 1952)
19. Frenkel, A. W., *J. Am. Chem. Soc.*, **76**, 5568 (1954)
20. Frenkel, A. W., *J. Biol. Chem.*, **222**, 823–34 (1956)
21. Frenkel, A. W., *Ann. Rev. Plant Physiol.*, **10**, 53–70 (1959)
22. Füchtbauer, W. and Simonis, W., *Naturwissenschaften*, **45**, 519 (1958)
23. Goodman, M., Bradley, D. F., and Calvin, M., *J. Am. Chem. Soc.*, **75**, 1962–67 (1953)
24. Grube, K. H., *Planta*, **42**, 279–303 (1953)
25. Jagendorf, A. T., *Brookhaven Symposia in Biol.*, **11**, 236–57 (1958)
26. Jagendorf, A. T. and Avron, M., *J. Biol. Chem.*, **231**, 277–90 (1958)
27. Kamen, M. D., *Research in Photosynthesis*, 149–63 (Interscience Publishers, New York, N.Y., and London, England, 1957)
28. Kandler, O., *Z. Naturforsch.*, **5b**, 423–37 (1950)
29. Kandler, O., *Z. Naturforsch.*, **9b**, 625–44 (1954)
30. Kandler, O., *Z. Naturforsch.*, **12b**, 271–80 (1957)
31. Kok, B., *Enzymologia*, **13**, 1–56 (1948)
32. Latzko, E. and Mechsner, Kl., *Naturwissenschaften*, **45**, 247–48 (1958)
33. Lynen, F. and Koenigsberger, F., *Ann. Chem. Liebigs*, **573**, 60–84 (1951)
34. Mechsner, Kl., *Biochim. et Biophys. Acta*, **33**, 150–58 (1959)
35. Mudd, J. B., *Nature*, **183**, 900–1 (1959)
36. Racker, F., *Nature*, *175*, 249–51 (1955)
37. Ruben, S., *J. Am. Chem. Soc.*, **65**, 279–82 (1943)
38. San Pietro, A., *Brookhaven Symposia in Biol.*, **11**, 262–69 (1958)
39. San Pietro, A. and Lang, H. M., *Science*, **124**, 118–19 (1956)
40. Simonis, W. and Grube, K. H., *Z. Naturforsch.*, **7b**, 194–96 (1952)

41. Simonis, W. and Kating, H., *Z. Naturforsch.*, **11b**, 165–72 (1956)
42. Simonis, W. and Weichart, G., *Z. Naturforsch.*, **13b**, 55–57 (1958)
43. Strehler, B. L., *Arch. Biochem. Biophys.*, **43**, 67–79 (1953)
44. Trebst, A., Tsujimoto, H. Y., and Arnon, D. I., *Nature*, **182**, 351–55 (1958)
45. Vernon, L. P., *Biochim. et Biophys. Acta*, **32**, 589–91 (1959)
46. Warburg, O. and Krippahl, G., *Svensk Kem. Tidsk.*, **69**, 143–56 (1957)
47. Wassink, E. C. and Rombach, J., *Koninkl. Ned. Akad. Wetenschap. Proc., Ser. C.*, **57**, 493–97 (1954)
48. Wessels, J. S. C., *Rec. trav. chim.*, **73**, 529–36 (1954)
49. Wessels, J. S. C., *Biochim. et Biophys. Acta*, **25**, 97–100 (1957)
50. Wessels, J. S. C., *Biochim. et Biophys. Acta*, **29**, 113–23 (1958)
51. Whatley, F. R., Allen, M. B., and Arnon, D. I., *Biochim. et Biophys. Acta*, **16**, 605–6 (1955)
52. Zöllner, N., *Z. physiol. Chem. Hoppe-Seyler's*, **291**, 157–59 (1952)

BIOSYNTHETIC PATHWAYS OF AROMATIC COMPOUNDS[1,2]

By A. C. Neish

Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada

Introduction

This review is concerned with the formation and interconversion of aromatic compounds in plants. It is restricted to aromatic compounds of the benzenoid type and excludes pyridine compounds. Lack of space also prohibits a discussion of the indole compounds. Attention will be focused on biosynthetic pathways revealed by use of the tracer technique.

The higher plants form a wide variety of benzenoid compounds. Some of these, like the aromatic amino acids, are universally distributed in nature. Others are classed as secondary growth substances (1) and are found in restricted taxonomic groups, sometimes in only a few species. Aromatic secondary growth substances include such substances as lignin, lignans, flavonoids, and phenolic glucosides [Geissman & Hinreiner (2)]. In addition, many alkaloids are benzenoid compounds (3). The wide variety of these aromatic substances has aroused much interest concerning both their origin and their possible function in the plant in which they occur. Since many of them are restricted to a few species, and are not found at all in animals or microorganisms, they can not be essential for life.

As plants evolved from simpler organisms, a biochemical as well as morphological evolution can be imagined. Mutations upsetting the delicate metabolic balance could occur and result in the accumulation of a new product. In microorganisms this product could be excreted into the medium but in a higher plant it would more likely be retained in the tissues. In some instances this new product might contribute to the survival of the plant. It could be a substance which would attract insects to the flowers, thus ensuring pollination, or it could have properties that would repel harmful insects [Fraenkel (4)]. Alternatively, a substance making the plant less susceptible to attack by parasitic microorganisms or better able to resist adverse environmental conditions might accumulate. It is not the purpose of this review to consider the functions of secondary growth substances but rather the mechanism of their biosynthesis. However, the concept of biochemical evolution is useful in attacking the problems of biosynthesis.

The chemical structures of many aromatic secondary growth substances are known. Robinson (5) has made a number of deductions concerning the biogenesis of these substances in plants by considering their structural rela-

[1] The survey of literature pertaining to this review was concluded May 1, 1959.

[2] The following abbreviations will be used: PEP (phosphoenol-pyruvate); E-4-P (D-erythrose-4-phosphate); DHP (dehydrogenation polymer).

tionships. In the first Weizmann Memorial Lecture (5) he points out the need for confirming these theories by use of the newly-developed tracer method. Since then a number of tracer experiments, mainly with C^{14}-labelled compounds, have been carried out on the biosynthesis of aromatic compounds in plants. Some of Robinson's views were supported by these experiments and in addition new problems were raised.

The aromatic amino acids, or intermediates involved in their biosynthesis, may be expected to act as precursors of many of the benzenoid secondary growth substances of plants. This view is derived from the concept of a biochemical evolution and from a consideration of the structural relations of natural products. It has received support from the tracer experiments reviewed below.

It is reasonable to suppose that the universally occurring aromatic amino acids are made from carbohydrates following essentially the same pathway in higher plants as in microorganisms. A great deal is now known about the biosynthesis of aromatic amino acids in microorganisms. Recent work in this field as well as that on higher plants will be reviewed. A number of alternate pathways for benzenoid compound formation from non-benzenoid compounds will also be considered. Finally, experiments bearing on the formation of secondary growth substances from simpler benzenoid compounds will be discussed. In a review of this type it is difficult to avoid a multiplicity of structural formulae. In some instances the presentation has been simplified by the use of symbols, as illustrated in Figure 1, for certain groups.

Biosynthesis of Benzoid Compounds From Non-aromatic Compounds

Biosynthesis of aromatic amino acids in Escherichia coli *(the shikimic acid pathway).*—The outstanding researches of Davis and his collaborators with biochemical mutants of *E. coli* established a route for biosynthesis of the aromatic amino acids from carbohydrates. This work has been previously reviewed by Davis (6, 7) and only the most recent investigations on this pathway will be considered here.

The presently accepted pathway for biosynthesis of phenylalanine and tyrosine is shown in Figure 2. Early auxotrophic studies (6) with mutants revealed that dehydroquinic acid (III), dehydroshikimic acid (IV), and

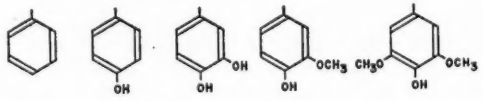


Fig. 1. Abbreviations used in structural formulae. R = phenyl, Rp = *p*-hydroxyphenyl, Rc = catechyl, Rg = guaiacyl, Rs = syringyl.

shikimic acid (V) were obligate intermediates in the formation of all the aromatic compounds required by *E. coli* for growth. It was also observed that quinic acid (II) could be utilized as an intermediate by *Aerobacter aerogenes*. However, the auxotrophic studies did not give much information about the biosynthesis of dehydroquinic acid from carbohydrates. This part of the scheme has been established by the tracer method and by studies on the enzymes found in cell-free extracts.

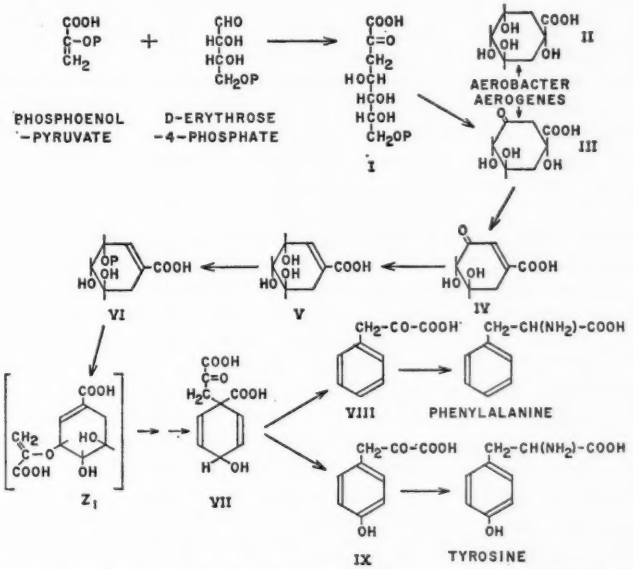


FIG. 2. Biosynthesis of phenylalanine and tyrosine in bacteria [see Davis (7)]. The symbol P used in a structural formula stands for orthophosphate at any stage of ionization.

Srinivasan *et al.* (8) determined the distribution of C^{14} in the shikimic acid accumulated by an *E. coli* mutant, after feeding glucose that had been labelled with C^{14} in various positions. They concluded that shikimic acid was formed by combining a four-carbon compound with a three-carbon compound, the latter being formed by glycolysis. Srinivasan, Katagiri & Sprinson (9) found that a cell-free extract of an *E. coli* mutant would catalyze the formation of 5-dehydroquinic acid (III) from D-erythrose-4-phosphate and phosphoenolpyruvate. Srinivasan & Sprinson (10) identified 2-keto-3-deoxy-D-arabo-heptonic acid-7-phosphate (I) as an intermediate in this reaction and concentrated the synthetase responsible for its production.

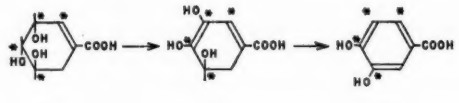
Weissbach & Hurwitz (11, 12) have also shown that a cell-free extract of *E. coli* formed a 2-keto-3-deoxyheptonic acid on addition of D-ribose-5-phosphate or D-erythrose-4-phosphate plus phosphoenol-pyruvate.

The carbon in the aromatic amino acids (Fig. 2) is thus derived from one mole of erythrose-4-phosphate and two moles of phosphoenol-pyruvate (6, 7). The second mole of phosphoenol-pyruvate is incorporated by reaction with 5-phosphoshikimic acid (VI) to give the compound Z_1 which then rearranges to form prephenic acid (VII). Prephenic acid serves as a branching point. It may be converted to either phenylpyruvic acid (VIII) or to *p*-hydroxyphenylpyruvic acid (IX) and these compounds give phenylalanine and tyrosine respectively, by transamination. Ghosh (13), and Schwink & Adams (14) have studied the latter stages of tyrosine formation and their results support Figure 2. There is no reason to believe that *p*-hydroxyphenylpyruvic acid is formed by hydroxylation of phenylpyruvic acid. Rather there are two ways of aromatizing" prephenic acid. One route gives phenylpyruvic acid and the other *p*-hydroxyphenylpyruvic acid.

The shikimic acid pathway in fungi.—The mold *Neurospora crassa* appears to utilize the shikimic acid pathway for biosynthesis of aromatic amino acids. Tatum *et al.* (15) obtained a mutant of this organism with a multiple nutritional requirement for aromatic amino acids. They found that this requirement could be satisfied by shikimic acid. Metzenberg & Mitchell (16) were unable to obtain any mutants which accumulated shikimic acid but they produced mutant strains which would accumulate 5-dehydroshikimic acid (IV), prephenic acid (VII) or protocatechuic acid (XII).

Gross (17) isolated a new enzyme (dehydroshikimic dehydrase) from a mutant strain of *N. crassa* which accumulated dehydroshikimic acid during growth. This enzyme catalyses the conversion of 5-dehydroshikimic acid (IV) to protocatechuic acid (XII). It is an inducible enzyme since it was not found in wild-type cells. Tracer studies have shown that the aromatization reaction catalyzed by this enzyme proceeds as shown in Figure 3. Gross (17) converted shikimic acid-3,4,5,6-C^{14} (X) to 5-dehydroshikimic acid by dehydroshikimic reductase. The enol form of dehydroshikimic acid (XI) is presumed to be the substrate for dehydroshikimic dehydrase. In any case the protocatechuic acid formed has the labelling pattern shown (see XII, Fig. 3). This was established by degradation to β-keto-adipic acid using *Pseudomonas fluorescens* enzymes (17).

It seems that *N. crassa* synthesizes its aromatic acids by a pathway



X XI XII

FIG. 3. Formation of protocatechuic acid by a *N. crassa* enzyme [see Gross (17)].

similar to that found in *E. coli* but it has not been proven yet that the pathways are identical. Other fungi have not been investigated to any great extent, although Bassett & Tanenbaum (18) have found that shikimic acid caused an increased yield of 6-methyl-salicylic acid (XIII) (see Fig. 5) in replacement experiments with *Penicillium patulum*.

The shikimic acid pathway in higher plants.—Higuchi (19) has summarized some of the reasons for believing that the shikimic acid pathway (Fig. 2) does function in higher plants. Shikimic acid is of widespread occurrence, and some enzymes of the above pathway have been found in higher plants [Mitsuhashi & Davis (20)]. Unfortunately it is not feasible to work with auxotrophic mutants of higher plants, as has been done with bacteria and fungi, so this pathway cannot be established in higher plants with the same degree of certainty. However, experiments using the tracer technique have shown that shikimic acid is readily converted to a variety of aromatic compounds in a number of plant species.

Brown & Neish (21) found that shikimic acid-C^{14} was converted readily to lignin by *Triticum vulgare* and *Acer negundo,* as measured by incorporation of C^{14} into the lignin-fraction yielding vanillin (Rg-CHO) and syringaldehyde (Rs-CHO) on nitrobenzene oxidation. Eberhardt & Schubert (22) found that shikimic acid was converted to lignin in sugar cane. When shikimic acid labelled mainly in carbons 1 and 6 was fed, the vanillin subsequently isolated was labelled in the same way. This experiment shows that the ring of shikimic acid was converted to a benzenoid ring without rearrangement, as would be expected if the scheme shown in Figure 2 applies to higher plants. McCalla & Neish (23) have found that generally-labelled shikimic acid, when fed to cutting of *Salvia splendens,* was converted to caffeic acid (Rc $-$ CH $=$ CH $-$ COOH) labelled only in the ring. The absence of C^{14} from the β-carbon of caffeic acid shows loss of the carboxyl carbon of shikimic acid during its conversion to this phenylpropanoid compound. This loss also occurs during formation of phenylpropanoid compounds in *E. coli* (Fig. 2). Shikimic acid-C^{14} was found to act as a good precursor of quercetin in *Fagopyrum tataricum* by Underhill, Watkin & Neish (24) and it was also converted to 3,4-dihydroxyacetophenone (Rc-CO-CH_3) in twigs of *Picea pungens* (25). Kosuge & Conn (26, 27) have shown that shikimic acid is converted readily to *o*-coumaric acid (isolated as its glucoside) by excised shoots of *Melilotus alba* and Brown, Towers & Wright (28) extended these observations to the sweet grass, *Hierochloë odorata*. Finally, it has been demonstrated that shikimic acid-C^{14} can be converted to both phenylalanine and tyrosine in plants representing three different families. McCalla & Neish (29) have established this conversion for *Salvia splendens* (Labiatae), and Gamborg & Neish (30) for *Triticum vulgare* (Gramineae) and *Fagopyrum tataricum* (Polygonaceae.)

Weinstein, Porter & Laurencot (31) found that quinic acid-C^{14} was converted to shikimic acid, phenylalanine, and tyrosine in young rose blooms.

This suggests that higher plants are able to convert quinic acid to shikimic acid as has been shown for *A. aerogenes* (Fig. 2).

There is good evidence that the phosphoenol-pyruvate (PEP) and D-erythrose-4-phosphate (E-4-P) required for the synthesis of shikimic acid (Fig. 2) can be formed in higher plants. The well established Embden-Meyerhof pathway produces PEP, while E-4-P can probably be formed as shown in Figure 4. The net reaction is:

$$\text{F-6-P} + 2\ \text{Gl-3-P} \longrightarrow 3\ \text{E-4-P}.$$

Since many terrestrial plants have a 20 to 30 per cent lignin content, this process may be responsible for metabolizing a large amount of hexose annually. Evidence for E-4-P formation in plants rests mainly on its being a substrate for the ubiquitous enzymes, transketolase, and transaldolase. These enzymes are believed to form E-4-P as a transitory intermediate during respiration by the pentose phosphate pathway (*32*, 33), and in operation of the photosynthetic carbon cycle (34, 35). It is necessary to postulate formation of E-4-P to explain the cyclic nature of these processes. Furthermore, Moses & Calvin (36) have direct evidence for formation of E-4-P during photosynthesis by *Chlorella*. However, it should be emphasized that

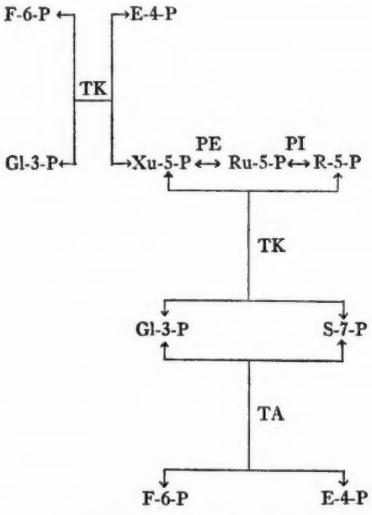


FIG. 4. Generation of D-erythrose-4-phosphate from hexose-phosphate. F-6-P = D-fructose-6-phosphate; Gl-3-P = D-glyceraldehyde-3-phosphate; Xu-5-P = D-xylulose-5-phosphate; Ru-5-P = D-ribulose-5-phosphate; S-7-P = Sedoheptulose-7-phosphate; E-4-P = D-erythrose-4-phosphate; TK = transketolase; TA = transaldolase; PE = phosphoribulo-epimerase; PI = phosphoriboisomerase.

production of E-4-P does not necessarily depend on the operation of either of these cycles. The enzymes of the Embden-Meyerhof pathway and those indicated in Figure 4 should suffice. In other words, there is no reason to believe that tissues synthesizing shikimic acid should be able to carry on either photosynthesis or respiration by the pentose phosphate pathway.

Biosynthesis of benzenoid compounds from acetate.—Collie (37, 38) made some ingenious suggestions many years ago concerning the origin of naturally occurring aromatic compounds from polyacetic acids. The latter are formed by head-to-tail condensations of acetic acid molecules. These theories have been developed further by other chemists, notably by Robinson (5), Birch (39) & Erdtman (40). The first biochemical evidence to support the theories was provided by Birch, Massy-Westropp & Moye (41) when they found that cultures of *Penicillium griseofulvin* formed 6-methyl salicylic acid (XIII) from acetate-1-C^{14} as shown in Figure 5A. Since then Birch and his collaborators have demonstrated that a number of aromatic compounds formed by fungi can be derived from acetate units condensed head-to-tail. These investigations will not be considered further here.

Studies on the biosynthesis of flavonoids have also shown that the acetate pathway operates in higher plants. This was established for quercetin (XIV) and cyanidin (XV) by the independent, simultaneous researches of four different laboratories. Watkin, Underhill & Neish (42) found that ring A of quercetin was formed from acetate by *Fagopyrum tataricum*. Geissman & Swain (43) made a similar observation with *Fagopyrum esculentum* and Shibata & Yamazaki (44) with *Fagopyrum cymosum*. Ring A became more heavily labelled in these experiments than did ring B when C^{14}-labelled acetate was fed. Grisebach (45) found that red cabbage seedlings formed

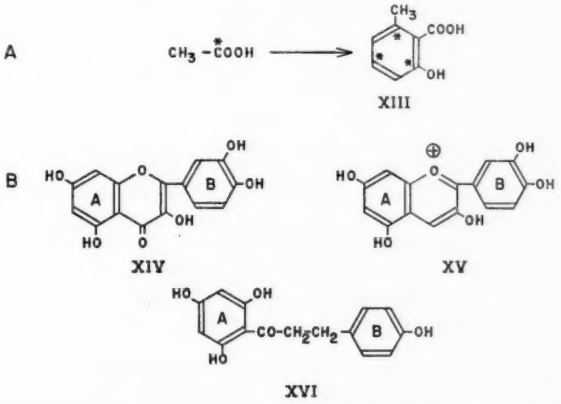


FIG. 5. Benzenoid compounds formed from acetate. Only ring A of the flavonoids comes from acetate; ring B is derived by the shikimic acid pathway.

ring A of cyanidin from acetate-1-C^{14} and demonstrated that the pattern of ring labelling was as expected from postulating a head-to-tail condensation of acetate molecules. Hutchinson, Taper & Towers (46) have added another C_6-C_3-C_6 compound to the list of products derived from acetate. Apple leaf discs converted acetate-2-C^{14} to phloretin (XVI), labelled mainly in ring A.

Other pathways for biosynthesis of benzenoid rings.—A number of routes for the biosynthesis of aromatic compounds, differing from the shikimic acid or acetate pathways, have been detected by the tracer method.

Goodwin & Treble (47) found that C^{14}-labelled acetoin (XVII) was converted to riboflavin (XVIII) in cultures of *Eremothecium ashbyii.* The C^{14} was found chiefly in ring A and its methyl groups (Fig. 6A). A plausible route for formation of riboflavin from acetoin was suggested.

Inositol (XIX) has often been considered as a possible precursor of aromatic compounds in plants. However, Weygand *et al.* (48) found that *meso*-inositol-C^{14} was not converted to catechins in tea leaves or to cyanidin in red cabbage seedlings. The mold *Phycomyces blakesleeanus* did not utilize inositol for formation of protocatechuic acid (XII) or gallic acid. However, *Pseudomonas beijerinckii* converted the labelled inositol to tetrahydroxybenzoquinone (XX) (Fig. 6B).

The phenazine ring found in pyocyanine (XXI) and related pigments produced by a few species of bacteria, is probably formed by a pathway distinct from any mentioned so far. Blackwood & Neish (49) compared a number of C^{14}-labelled compounds as precursors of pyocyanine in cultures of *Pseudomonas aeruginosa* and found glycerol and dihydroxyacetone were superior to all other substances tested. These included glucose, acetate, and phenylalanine. Oxidative demethylation of the pyocyanine formed from glycerol-1,3-C^{14} gave 1-hydroxyphenazine which retained most of the C^{14}, thus proving glycerol contributed to the formation of phenazine nucleus (Fig. 6C).

There is a possibility that plants are able to form aromatic rings by dehydrogenation. Although such reactions have not been demonstrated in plants, biochemical analogies are known. Mitoma, Posner & Leonard (50) have obtained a microsomal fraction from guinea pig liver which catalyses the conversion of hexahydrobenzoic acid (XXII) to hippuric acid (XXIII) (Fig. 6D), and Ayengar *et al.* (51) have found a TPN-linked diol dehydrogenase in rabbit liver which catalyses the oxidation of 3,5-cyclohexadiene (XXIV) to catechol (XXV) (Fig. 6E). A comparison of the structures of menthol (XXVI) and thymol (XXVII) suggests that aromatization by dehydrogenation may occur in plants (Fig. 6F), but this has not been established by the tracer method or by enzyme studies.

Biosynthesis of Aromatic Compounds From Aromatic Precursors

General.—Although some aromatic compounds in plants are formed from non-benzenoid precursors it is probable that most, in the final stages,

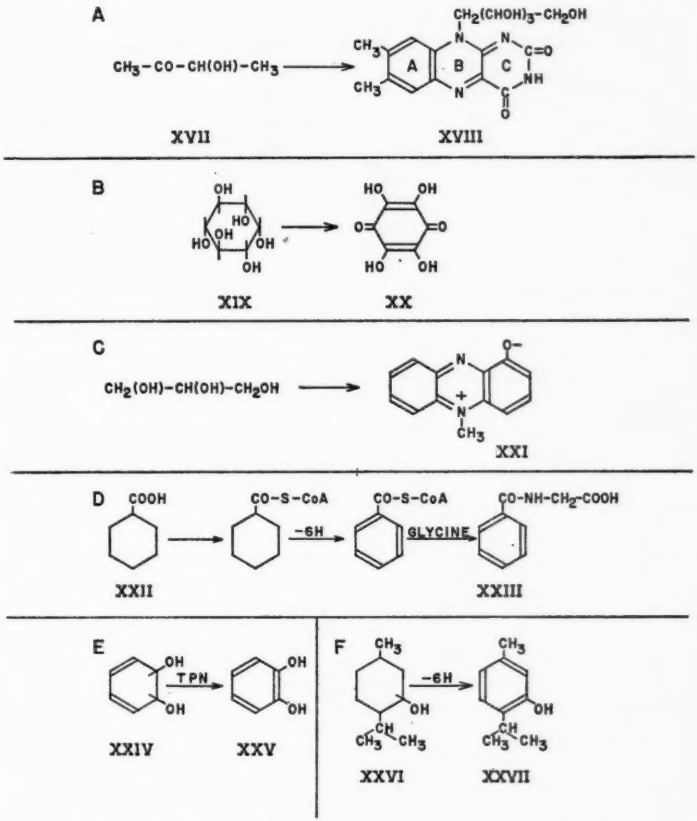


FIG. 6. Some biochemical pathways for formation of aromatic compounds.

are formed from other aromatic compounds. In other words, a few aromatic compounds are formed from carbohydrates, with the remainder generated by a complex series of interconversions. The shikimic acid pathway, discussed above, is probably an important route for formation of aromatic C_6-C_3 and C_6-C_1 compounds in plants. Compounds that might be expected to arise by this pathway are protocatechuic acid, *p*-hydroxybenzoic acid, anthranilic acid, *p*-aminobenzoic acid, phenylpyruvic acid, and *p*-hydroxyphenylpyruvic acid (6, 7). As far as is known, most of the aromatic secondary growth substances must be formed from these compounds and from acetate.

The phenylpropanoid (i.e., C_6-C_3) compounds of plants constitute a large

and diverse group. This is now understandable since two important products of the shikimic acid pathway have the phenylpropanoid structure. There are several ways for getting phenylpropanoid compounds from other aromatic compounds. Higuchi (19, 52) has suggested that phenylpropanoid lignin precursors may be formed from C_6-C_1 aldehydes by reactions similar to the carboligase reaction discovered in fermenting yeast by Carl Neuberg. A new enzyme, phenylserine aldolase, recently found in liver by Bruns & Fiedler (53) catalyses a reversible condensation of benzaldehyde derivatives with glycine, as illustrated by the following:

$$R-CHO+CH_2(NH_2)-COOH \leftrightharpoons R-CH(OH)-CH(NH_2)-COOH.$$

If this enzyme exists in plants it might provide an alternate route for formation of C_6-C_3 compounds, as well as a route for formation of benzaldehyde derivatives from C_6-C_3 compounds. Present evidence favors the view that phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid, formed *via* the shikimic acid pathway, are the aromatic precursors of a great many plant products. This raises a multitude of questions concerning the pathways involved in their transformation to end-products such as lignin, flavonoids, phenolic acids, phenolic glucosides, coumarins, alkaloids, etc. The remainder of this review attempts to answer some of these questions.

Early reactions in conversion of simple phenylpropanoids to lignin and related compounds.—The basic idea has been advanced by Hibbert (54) that simple precursors of lignin are interconverted by reactions analogous to those in respiration of carbohydrates. It is unlikely, however, that Hibbert's ketones are intermediates between the aromatic keto acids (VIII and IX) and lignin. The phenolic cinnamic acids are more probable intermediates since they are known to be widely distributed in plants [Bate-Smith (55), Kremers (56)], and also because they are highly ionized under physiological conditions (7).

Results of tracer experiments support the idea that phenolic cinnamic acids are important intermediates in the formation both of lignin and of other secondary growth substances. Reznik (57) postulated these acids to be common precursors of many aromatic plant constituents. A similar conclusion was reached independently by the group in Saskatoon based on a series of comparative studies on the efficiency of several labelled compounds as precursors of lignin and other aromatic substances. The labelled compounds were fed to plants or excised shoots. The plant material was allowed a certain time for metabolism, then worked up, and the specific activity of the end-product (lignin, quercetin, etc.) measured. By means of numerous parallel experiments a number of C^{14}-labelled compounds were compared in efficiency as precursors of several end-products. It was reasoned that precursors readily forming a given end-product might have a close metabolic relation to each other. If they were related structurally this conviction was strengthened and tracer experiments were carried out to see if one precursor could be converted to the other. Experiments of this type have produced the

scheme shown in Figure 7 [see Brown, Wright & Neish (58), McCalla & Neish (23)]. Some of the evidence on which Figure 7 is based has already been reviewed above, i.e., the experiments showing that shikimic acid and quinic acid can function as precursors of aromatic amino acids and other compounds of the phenylpropanoid type in plants. Further evidence in support of this scheme follows.

All compounds shown as intermediates in Figure 7 have been fed to excised shoots of wheat and maple and found to be readily converted to lignin,

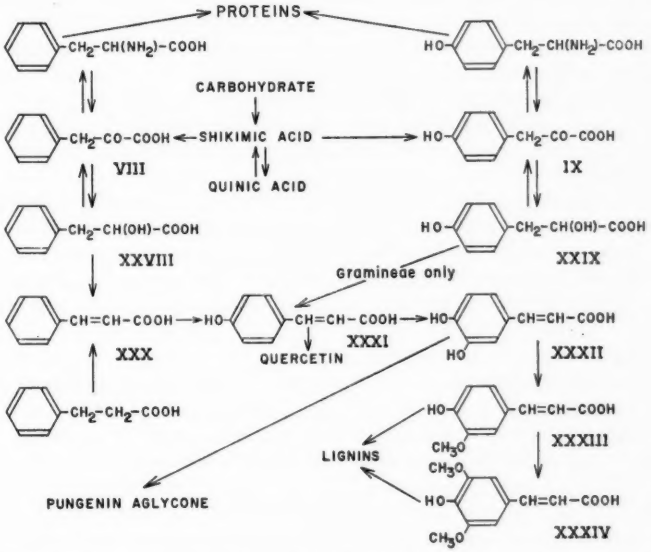


FIG. 7. Early reactions leading to formation of lignin and related compounds.

as measured by the vanillin (Rg-CHO) and syringaldehyde (Rs-CHO) obtained by nitrobenzene oxidation of the extractive-free plant material [Brown & Neish (21, 59, 60); Wright, Brown & Neish (61); Brown, Wright & Neish (58)]. In these experiments a number of interesting taxonomic differences were noted. Out of the 11 species representing 10 plant families, only two could readily convert tyrosine to lignin, although all species readily utilized phenylalanine as a lignin precursor. The species using tyrosine were *Triticum vulgare* and *Calamogrostis inexpansa,* both members of the family Gramineae and the only ones tested from that family. Species also varied in their ability to utilize the D and L forms of phenyllactic acid (XXVIII). Some preferred the *levo* isomer while others used both isomers to about the same extent (61).

All the phenyl compounds in Figure 7 were utilized for biosynthesis of the C_6(ring B) — C_3 portion of the quercetin molecule (XIV) in buckwheat (24). Tyrosine was not utilized although *p*-coumaric acid was a good precursor. This agrees with the observation that buckwheat cannot form lignin from tyrosine (60) and suggests that tyrosine cannot act as a precursor of *p*-coumaric acid in buckwheat.

The phenolic cinnamic acids of Figure 7 all occur as depsides in the stems of *Salvia splendens*. They are readily biosynthesized from L-phenylalanine and the kinetics of these transformations could be interpreted by assuming that hydroxylation of the ring and O-methylation proceeded in a stepwise manner. This was investigated by feeding experiments with labelled *trans*-cinnamic acid (XXX), *p*-coumaric acid (XXXI), caffeic acid (XXXII), ferulic acid (XXXIII), and sinapic acid (XXXIV). Each precursor was easily converted to a more highly substituted member of the series but such conversions did not appear readily reversible. Tyrosine was not effective as a precursor [McCalla & Neish (23)].

The 3-glucoside of 3,4-dihydroxyacetophenone (pungenin) is a major constituent of the leaves of Colorado spruce (62, 63). The biosynthesis of the aglycone (Rc-CO-CH_3) has been investigated by the tracer method [Neish (25)]. Phenylalanine and caffeic acid (XXXII) were the best precursors of this C_6-C_2 compound while tyrosine, dopa, acetate, and several C_6-C_2 compounds were not utilized. Cinnamic acid, phenyllactic acid (XXVIII) and *p*-coumaric acid (XXXI) were fairly good precursors.

Gamborg & Neish (30) found that phenylpyruvic acid and phenyllactic acid were incorporated into the bound phenylalanine of young wheat or buckwheat shoots as readily as was phenylalanine itself. The corresponding *p*-hydroxyphenyl compounds (Rp-CH_2-CO-COOH and Rp-CH_2-CH(OH)-COOH) were freely incorporated into the tyrosine of both species (30, 58). Thus the α-keto and α-hydroxy acids of Figure 7 are readily converted to the corresponding α-amino acids. There was no conversion of *p*-hydroxyphenyl derivatives to phenylalanine, and a relatively small utilization of the phenyl compounds for tyrosine synthesis. Neither cinnamic acid nor *p*-coumaric acid were converted to the amino acids.

The scheme in Figure 7 is a working hypothesis and is supported mainly by tracer experiments with living plants. Studies on isolated enzyme systems are needed. The intermediates shown may exist as activated forms and react as such, at least for some of the reactions, since their efficiencies as precursors do not fall into the order expected. For example, L-phenylalanine is often found to be the best precursor of a particular end-product although it occurs far back in the scheme. This behaviour can be explained if activated forms such as coenzyme A esters or mixed acid anhydrides with adenylic acid are the true intermediates. The rate of utilization for the compound fed might then be governed by its rate of activation. All the intermediates in Figure 7 cannot be arranged in order by considering their efficiency as precursors of a given end-product. The present arrangement is partly based

on structural considerations permitting one intermediate to be converted to another by simple chemical changes for which numerous biochemical analogies may be cited. Brown *et al.* (58) have suggested that the failure of many species to use tyrosine for synthesizing lignin and related compounds, is due to their inability to dehydrate *p*-hydroxyphenyllactic acid (XXIX) (Fig. 7). However, it is not certain that either cinnamic acid or *p*-coumaric acid is formed by dehydration of the correspounding α-hydroxy acid. They might be formed by removing the elements of ammonia from the corresponding α-amino acids. The tracer studies reviewed above do not distinguish between these two possible routes.

Acerbo, Schubert & Nord (64) found *p*-hydroxyphenylpyruvic acid (IX) to be converted to lignin in high yields in sugar cane. Nord & Schubert (65) have suggested that this keto acid is a central intermediate in lignification. However, Billek (66) has reported that *p*-hydroxyphenylpyruvic acid was not converted to lignin in spruce. This apparent polemic is easily resolved by reference to Figure 7, since sugar cane is a member of the Gramineae and spruce is not. In other words, these divergent results support the scheme for interconversion of phenylpropanoid compounds. It is obvious that too few species have been tested to permit a sharply-defined grouping but current evidence favors the view that only members of the Gramineae can utilize tyrosine and the acids IX and XXIX for the formation of lignin.

The extensive literature dealing with enzymic hydroxylations of benzenoid nuclei cannot be discussed here. Methoxyl groups are formed by enzymic methylation of hydroxyl groups and Byerrum *et al.* (67) have shown that methoxyl groups of lignin can be formed in barley and tobacco plants by transmethylation from methionine. Presumably the methoxyl groups of the intermediates shown in Figure 7 are derived in this manner.

Caffeic acid.—Caffeic acid (XXXII) is widely distributed in nature in free form and as depsides such as chlorogenic acid (55, 56). Some experiments on the biosynthesis of caffeic acid have been cited above in discussing the shikimic acid pathway in plants and the evidence supporting the scheme in Figure 7. However, the first tracer investigation of the biosynthesis of this compound was made by Geissmann & Swain (43). They found that phenylalanine-α-C^{14} was converted to caffeic acid by *Nicotiana tabacum* without rearrangement of the carbon skeleton. Reid (68) observed that young tobacco shoots formed the caffeic acid part of chlorogenic acid much more readily from phenylalanine than from acetate or phenylacetic acid. Reznik & Urban (69) have shown that ferulic acid-β-C^{14} can be converted to chlorogenic acid and other Rc-compounds in wheat, sunflower, and corn, and in red cabbage seedlings. It has also been found, as mentioned above, that L-phenylalanine, (-)-phenyllactic acid, cinnamic acid and *p*-coumaric acid are all good precursors of caffeic acid in *Salvia splendens* (23).

These results conform with the scheme in Figure 7 except for the finding of Reznik & Urban that demethylation of hydroxyl groups may occur quite readily. In this connection it should be mentioned that such demethylations

have been demonstrated in other systems. Shimazono & Nord (70) found that *Lentinus lepideus* could convert methyl *p*-methoxycinnamate to methyl *p*-coumarate, and Axelrod & Szara (71) have obtained an enzyme system from rabbit liver which could convert metanephrine (Rg-CH(OH)-CH_2-NH-CH_3) to epinephrine (Rc-CH(OH)-CH_2-NH-CH_3).

Lignin.—Some idea of the complex nature of lignin may be gained by reading recent reviews dealing with lignin from the viewpoint of the organic chemist (72 to 75), the biochemist (19, 65, 74, 75), the plant physiologist (76, 77, 78) and the plant morphologist (77, 79). Kratzl (80) summarized the conclusions of a recent symposium at which lignin was discussed from several different viewpoints.

A number of difficulties involved in biochemical investigations of lignin stem from the fact that the structure of lignin is only partially understood. Indeed, no one knows how many different kinds of lignin exist. It is probable that lignin, like hemicellulose, represents a group of related compounds varying from one taxonomic group to another and possibly from one tissue to another in the same plant. In the course of evolution the ability to make a guaiacyl lignin was acquired first with the more complex angiosperm lignins developed later. Histochemical tests have demonstrated that lignin of the primary xylem of angiosperms is like the guaiacyl lignin of conifers while the more complex lignins, containing both syringyl (Rs) and guaiacyl (Rg) nuclei, are formed only in cells differentiating from the cambium. It appears that even in lignification "ontogeny recapitulates phylogeny" [Manskaja (79); Wardrop & Bland (77)].

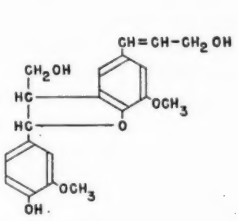
Most experts agree that lignin is a polymer constructed from phenylpropanoid units. This conclusion suggests origin by the shikimic acid pathway and there is no good reason to believe the acetate pathway contributes significantly to lignin formation. Labelled acetate was not incorporated into lignin of wheat, maple, or spruce as measured by isolation of vanillin or syringaldehyde (25, 81), or into wheat or maple lignin as measured by isolation of phenylpropanoid hydrogenolysis products (81).

Tracer studies on lignin biosynthesis, discussed previously in relation to Figure 7, support the opinion that lignin is phenylpropanoid since C_6-C_1 and C_6-C_2 compounds were generally found inferior to C_6-C_3 compounds as lignin precursors (21, 58, 59, 60, 61). These tracer studies were designed primarily to establish metabolic relations between simple aromatic compounds; therefore only C_6-C_1 decomposition products of lignin (i.e., Rg-CHO and Rs-CHO) were isolated. In a more recent study with wheat and maple, Brown & Neish (81) also isolated hydrogenolysis products with the C_6-C_3 structure, i.e., Rg-CH_2-CH_2-CH_2OH and Rs-CH_2-CH_2-CH_2OH. The same conclusions were generally reached whether the easily-isolable C_6-C_1 aldehydes or the hard-to-get hydrogenolysis products were taken to represent lignin.

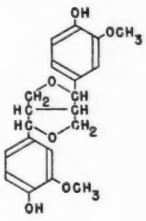
Freudenberg (74) believes that conifer lignin is formed from coniferyl alcohol (Rg − CH = CH − CH_2OH). This alcohol is found as its glucoside (coniferin) in the cambial sap of spruce. Freudenberg & Niedercorn (82)

have shown that phenylalanine-C^{14} acts as a precursor of coniferin aglycone and of lignin in young spruce saplings. Reichert, working in Freudenberg's laboratory (83), found that ferulic acid was converted to spruce lignin as measured by isolating C_6-C_3 ethanolysis products such as Rg-CO-CO-CH_3. Cinnamic acid is also converted to spruce lignin (25). These results support the view that ferulic acid, produced as indicated in Figure 7, is reduced to coniferyl alcohol which is then polymerized to lignin. There is ample evidence that coniferyl alcohol, labelled specifically in the $-CH=CH-CH_2OH$ side chain, can be converted to spruce lignin without rearrangement of the carbon skeleton. This has been demonstrated by Freudenberg's group (74, 83) and by Kratzl and his colleagues (84 to 87). The coniferyl alcohol was fed as coniferin in their experiments and lignin degradation products representing all the carbons of the C_6C_3 monomers were isolated.

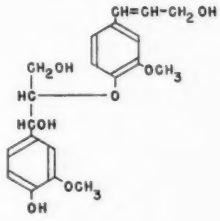
Freudenberg (74) concluded that conifer lignin is formed by oxidative polymerization of coniferyl alcohol. This view is supported by *in vitro* studies on the behavior of coniferyl alcohol in dilute aqueous solutions in the presence of a laccase. This system produces a water-insoluble dehydrogenation polymer (DHP) with properties remarkably similar to those of spruce milled-wood lignin. Water-soluble dimers were also isolated. The main dimers obtained were dehydrodiconiferyl alcohol (XXXV), DL-pinoresinol (XXXVI) and guaiacylglycerol-β-coniferyl ether (XXXVII) (see Fig. 8). Continued action of the enzyme converted these dimers to a



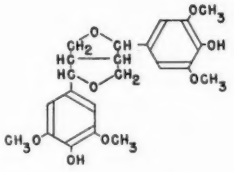
XXXV



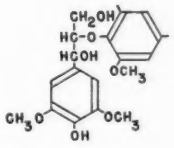
XXXVI



XXXVII



XXXVIII



XXXIX

FIG. 8. Structures relevant to lignin formation.

DHP. All of these products were optically inactive although several asymmetric centers are present. Freudenberg (88) and Adler (89) have discussed the mechanism of this polymerization. The function of the enzyme is to produce a free radical by dehydrogenation. The DHP is then formed by spontaneous reactions not under enzymic control. This explains why the products are optically inactive and also why the laccase may be replaced by other enzymes [see Higuchi (90)] or even by cupric ions.

Freudenberg (74) believes the same processes operate during lignification of cells differentiated from the cambium. This would explain why no one has ever demonstrated optical activity in a lignin preparation. Although many biochemists may dislike the suggestion that a major natural product is produced by "laboratory reactions" it must be admitted that the lack of optical activity by lignin strongly supports this view. There is a reasonable doubt concerning this conclusion, however, since a complex polymer such as lignin could have optical activity at quite a low level due to internal compensation. Most lignin preparations are denatured products which form colored or colloidal solutions not suitable for accurate polarimetric measurements. Furthermore it is difficult to obtain a simpler lignin preparation by chemical degradation without using procedures likely to cause racemization. It might be concluded that it has not yet been proven whether or not lignin is optically active in its natural state. In this connection it should be pointed out that the naturally occurring lignans [see Erdtman (91)] are all optically active, some being *dextro*-rotatory and others *levo*. This group of compounds represents a number of different ways used by nature to form dimers of C_6-C_3 compounds. Pinoresinol (XXXVI) obtained from the wound resin of spruce cambium is optically active. If Freudenberg's theory (74) is accepted unconditionally then spruce produces DL-pinoresinol for lignification and *dextro*-pinoresinol when wounded.

Freudenberg's theory also includes angiosperm lignin. It is believed to be formed by copolymerization of coniferyl alcohol (Rg $-$ CH $=$ CH $-$ CH_2OH) and sinapyl alcohol (Rs $-$ CH $=$ CH $-$ CH_2OH). Model experiments with laccase or copper ions showed that sinapyl alcohol will not produce a DHP by itself since the reaction stops after syringaresinol (XXXVIII) is formed in high yields. However, a mixture of sinapyl alcohol and coniferyl alcohol will form a DHP in the model system. Kratzl & Buchtela (92) made this mixed polymer using laccase, and showed that it would yield ethanolysis products with the syringyl group, e.g., Rs-CO-CO-CH_3. This indicates that their DHP probably contains a β-aryl ether of the type XXXIX, since syringaresinol would not form Hibbert's ketones and strengthens the idea that angiosperm lignin originates by oxidative polymerization of a mixture of coniferyl and sinapyl alcohols. In addition, this view is supported by the observation that lignins from natural sources which contain syringyl nuclei also contain guaiacyl nuclei. *p*-Coumaryl alcohol (Rp $-$ CH $=$ CH $-$ CH_2OH) will form a DHP and it is believed to be a precursor of those angiosperm lignins given some Rp-CHO on oxidation.

It is possible that syringyl groups are formed by introducing methoxyls

into guaiacyl lignin after some polymerization has occurred. Stone, Blundell & Tanner (93) measured the syringaldehyde and vanillin yields obtained by oxidizing wheat plants at different stages of growth. The proportion of syringaldehyde increased as the plants aged. Higuchi (52) found a similar change in bamboo, where the molar ratio Rs-CHO/Rg-CHO changed gradually from 0.4 for the young tips to 2.0 for the mature wood. These results might be explained by methoxylation of guaiacyl lignin, or by increased incorporation of syringyl monomers, in older plants. Brown & Neish (81) observed that sinapic acid-β-C^{14}, fed to excised twigs of *Acer negundo,* formed a lignin which gave radioactive Rs-CHO on nitrobenzene oxidation but inactive Rs-CH_2-CH_2-CH_2OH on hydrogenolysis. The sinapic acid was not fixed as a simple ester. It may have been reduced and incorporated by a syringaresinol type of linkage or some type other than the β-aryl ether type, since a unit of the type XXXIX would be expected to form Rs-CH_2-CH_2-CH_2OH on hydrogenolysis. In these experiments ferulic acid-β-C^{14} was converted to a lignin which gave Rs-CH_2-CH_2-CH_2OH, Rg-CH_2-CH_2-CH_2OH, Rs-CHO, and Rg-CHO, all labelled with C^{14}. This may be explained by assuming ferulic acid reduction to coniferyl alcohol, then incorporation of the coniferyl alcohol into lignin by β-aryl ether linkages (see XXXVII), and finally introduction of methoxyl groups into the nuclei of this guaiacyl lignin. There are other possible explanations. It is true that Kratzl & Buchtela (92) have a good model for formation of aryl ether linkages from sinapyl alcohol but it has not been proven that this represents the only way angiosperm lignin may be formed in the plant. Reasons still exist for believing that methoxyl groups may be introduced into the guaiacyl nuclei at the dimer stage or even much later.

In summation it may be said that lignin is a product of the shikimic acid pathway. Ferulic acid (XXXIII), formed as indicated in Figure 7, is probably reduced to coniferyl alcohol. This alcohol may be stored temporarily, as the glucoside, in a few species such as spruce. An oxidative polymerization of coniferyl alcohol may then form guaiacyl lignin as postulated by Freudenberg (74). The onset of lignification might be determined by the redox systems in the cells as suggested by Higuchi (90), and indole acetic acid may be a controlling factor (77, 78). Actual polymerization is probably initiated by a laccase or peroxidase and may then proceed by spontaneous chemical reactions or possibly under closer enzymic control than achieved in model systems. In order to help decide this point a definite answer is needed to the question: Is undenatured lignin optically active? The lignins of angiosperms may be derived by oxidative copolymerization of coniferyl alcohol, sinapyl alcohol, and (sometimes) *p*-coumaryl alcohol. There is also a possibility that syringyl groups may be formed by methoxylation of the nuclei of guaiacyl lignin, as suggested in older theories of lignin formation.

Flavonoids.—There is not a great deal to add to Bogorad's recent review of this field (94). The early tracer experiments have established that ring B of quercetin (XIV) and the attached three carbons of the heterocylic ring

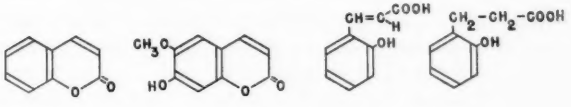
originate from a C_6-C_3 unit. Ring A is derived from acetate as discussed above. Thus Robinson's suggestion (5) that flavonoids may originate by condensation of a $C_6(B)$-C_3 unit with a $C_6(A)$ unit has received some experimental support. This dual origin of flavonoids, from both the shikimic acid and the acetate pathways, has been established for quercetin (XIV) (24, 42, 43, 44), cyanidin (XV) (45), and phloretin (XVI) (46) (see Fig. 5). Erdtman (40) has postulated a similar origin for pinosylvin and other stilbene derivatives on structural grounds.

Grisebach (95) has found that phenylalanine contributed to ring B but not to ring A of cyanidin, in red cabbage seedlings. Coniferin-β-C^{14} was not utilized as a precursor of cyanidin although it formed an unidentified compound with the properties of a flavone. Isotope competition experiments (96) failed to show that triacetic acid (CH_3-CO-CH_2-CO-CH_2-COOH) or its lactone were good precursors of ring A of cyanidin; acetate appears to be superior. Dim red light stimulated the production of cyanidin in red cabbage seedlings without affecting the rate of production of the $C_6(B)$-C_3 precursor. Red light appeared to stimulate formation of ring A precursors in the plant.

Comte *et al.* (97) found cinnamic acid could be incorporated into the flavones by young wild cherry trees. A substance with the properties of a catechin was also formed. Zaprometov (98) investigated formation of catechins following photosynthetic assimilation of $C^{14}O_2$ in the tea plant. The catechins were labelled to about the same extent whether whole plants or excised shoots were used, but catechin synthesis did not occur in detached mature leaves. This result agrees with a previous observation on the formation of quercetin in buckwheat (42). It was concluded that the young actively-growing shoots of the tea plant are the main sites of catechin synthesis. Young shoots of Georgian tea were found to convert a large fraction of photosynthetically assimilated $C^{14}O_2$ to catechin [see Kursanov & Zaprometov (99)].

In general it may be concluded that there has been no major advance in theories of flavonoid biogenesis since publication of Bogorad's review (94). Earlier work has been confirmed and extended. Phloroglucinol-C^{14} fed to plants was not incorporated into quercetin (100) or phloretin (46). Acetate is still the best known precursor of ring A. The best $C_6(B)$-C_3 precursors of quercetin found by Watkin & Neish (100) are L-phenylalanine, phenylpyruvic acid, (-)-phenyllactic acid, and *p*-coumaric acid. In buckwheat (30) *p*-coumaric (XXXI) acid is formed irreversibly from the other precursors so it must enter quercetin by a route not involving phenylpyruvic acid as an intermediate. Thus, *p*-coumaric acid is probably the C_6(ring B)-C_3 precursor of quercetin although an alternate pathway from phenylpyruvic acid is not excluded.

Coumarins.—The biosyntheses of coumarin (XL) and scopoletin (XLI) (Fig. 9) have both been investigated by the tracer technique. Since these compounds are the lactones of the *cis* forms of *o*-hydroxy-cinnamic acid and *o*-hydroxy-ferulic acid respectively, they might be expected to originate from



XL XLI XLII XLIII

FIG. 9. Coumarins and related compounds.

the *trans*-cinnamic acid (XXX) and ferulic acid (XXXIII) of Figure 7. The tracer investigations support this view.

Reznik & Urban (69) found that ferulic acid was converted to scopolin (scopoletin glucoside) when fed to leaves of wheat, corn, and sunflower plants. Reid (68) has shown L-phenylalanine to be superior to acetate, phenylacetic acid, or $C^{14}O_2$ as a precursor of scopoletin in the tobacco plant. These results can be explained by assuming that ferulic acid, produced as shown in Figure 7, is hydroxylated in the *ortho* position and then isomerized to scopoletin.

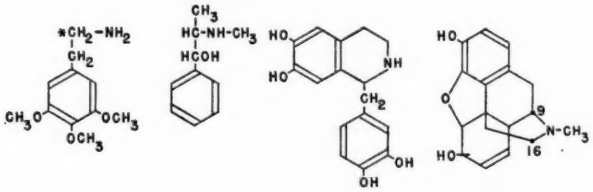
Kosuge & Conn (26, 27) have studied the formation and metabolism of coumarin in *Melilotus alba*. This species also contains *o*-coumaric acid (XLII) and its glucoside. Labelled coumarin was formed when the plants were activated photosynthetically by $C^{14}O_2$. The C^{14} incorporated into coumarin was later metabolized. The *o*-coumaric acid, fed to excised shoots, was converted mainly to *o*-coumaryl glucoside but also to melilotic acid (XLIII) and its glucoside. When *trans*-cinnamic acid, phenylalanine, or shikimic acid were fed they are converted to *o*-coumaric acid with less dilution of C^{14} than when glucose-C^{14} was fed, while carbon from acetate-C^{14} was incorporated less readily than that from glucose. Coumarin-C^{14} was converted to melilotyl glucoside, melilotic acid, and several unidentified compounds.

Brown, Towers & Wright (28) studied the biosynthesis of coumarin in *Melilotus officinalis* and in the sweet grass, *Hierochloë odorata*. The latter plant was found to be more convenient for laboratory studies. *H. odorata* formed coumarin more readily from *trans*-cinnamic acid, *o*-coumaric acid, phenylalanine, or shikimic acid than from acetate. This species also contains *o*-coumaryl glucoside, *p*-coumaric acid, ferulic acid, and melilotic acid. When cinnamic acid-β-C^{14} was fed to excised shoots it was converted rapidly to *o*-coumaryl glucoside and more slowly to coumarin. Most of the C^{14} incorporated was found in *o*-coumaryl glucoside. On further metabolism the specific activity versus time curve showed a steady rise in the specific activity of coumarin, up to 24 hr. after an initial lag, while the specific activity of the *o*-coumaryl glucoside remained fairly constant. The specific activities of *p*-coumaric acid and ferulic acid passed through a maximum at about 16 hr. These results suggest that in general cinnamic acid undergoes both *ortho* and *para* hydroxylation in *H. odorata*.

Coumarin thus appears to be a product of the shikimic acid pathway, formed as a result of *o*-hydroxylation of cinnamic acid.

Formation of C_6-C_2 compounds.—Tracer studies have been conducted to determine the origin of hordenine, mescaline, dhurrin aglycone, and pungenin aglycone. These C_6-C_2 compounds all appear to be products of the shikimic acid pathway. They are formed by processes involving loss of the terminal carbon of some C_6-C_3 compound.

Marion (101) has reviewed the work of his group on the biosynthesis of hordenine ($Rp\text{-}CH_2\text{-}CH_2N(CH_3)_2$) in barley seedlings. It appears that tyramine ($Rp\text{-}CH_2\text{-}CH_2NH_2$) is formed by decarboxylation of tyrosine and then undergoes a stepwise N-methylation to form hordenine [see also Brady & Tyler (102)]. Leete (103) has found that mescaline formed from tyrosine-α-C^{14} in *Anhalonium lewinii* was labelled as indicated in XLIV (Fig. 10). This suggests that it arises by decarboxylation of tyrosine or dopa followed by further hydroxylation of the ring and O-methylation.



XLIV XLV XLVI XLVII

FIG. 10. Alkaloids derived from phenylalanine or tyrosine.

Dhurrin aglycone ($Rp\text{-}CH(OH)\text{-}CN$) also appears to be a metabolic product of tyrosine. Dhurrin is found in *Sorghum vulgare* seedlings. Conn & Akazawa (104) found that these seedlings contain enzymes which hydrolyse dhurrin to glucose, hydrogen cyanide, and *p*-hydroxybenzaldehyde. Gander (105, 106) has studied the formation of dhurrin from labelled precursors in sorghum seedlings. Tyrosine-α-C^{14} was a much better precursor of the HCN from dhurrin than were phenylalanine-α-C^{14} or glycine-2-C^{14}. He obtained chromatographic evidence that $Rp\text{-}CH(OH)\text{-}CH(NH_2)\text{-}COOH$ was also formed from tyrosine, both in living plants and cell-free extracts.

The biosynthesis of pungenin aglycone ($R\text{-}CO\text{-}CH_3$) has been reviewed in the discussion of Figure 7. It is a product of the shikimic acid pathway, probably formed from caffeic acid, possibly by the route: $Rc - CH = CH - COOH \longrightarrow Rc - CH(OH) - CH_2 - COOH \longrightarrow Rc - CO - CH_2 - COOH \longrightarrow Rc - CO - CH_3$. This route seems the most direct from caffeic acid and conforms to Robinson's suggestion (5) that acetophenones are formed by decarboxylation of benzoylacetic acids. In contrast to the other C_6-C_2 compounds discussed above, pungenin aglycone is not a metabolic product of tryosine.

It appears likely that C_6-C_2 compounds are formed by decarboxylation of

C_6-C_3 compounds. In some cases this may be due to the action of amino acid decarboxylases but other types of decarboxylases are probably involved.

Ephedrine.—Shibata & Imaseki (107) administered N^{15}-labelled amino acids to ephedra plants growing in hydroponic culture. The isotopic nitrogen was incorporated into ephedrine (XLV) (Fig. 10) more easily from phenylalanine than from alanine. When methionine-methyl-C^{14} was fed, the C^{14} incorporated into the ephedrine was all in the N-methyl group; but when formate-C^{14} was administered, the C^{14} was incorporated almost equally into the N-methyl and C-methyl groups with relatively small amounts being found in other parts of the molecule (108, 109). Imaseki, Shibata & Yamazaki (110) found that ω-aminoacetophenone-carbonyl-C^{14} gave ephedrine labelled only in the carbon next the ring. This suggests that phenylalanine is decarboxylated to R-CH_2-CH_2NH_2 which then undergoes oxidation to R-CO-CH_2NH_2. Incorporation of methyl groups from methionine and formate then gives ephedrine. Leete (111) has shown that phenylalanine-β-C^{14} fed to excised shoots of *Catha edulis* gives rise to *dextro*-nor-pseudoephedrine labelled only in the carbon adjacent to the ring, as expected.

Ephedrine is thus a product of the shikimic acid pathway and appears to be metabolically related to the β-phenylethylamine alkaloids.

Morphine.—Alkaloid chemists believe that some of the more complex alkaloids such as the benzylisoquinoline alkaloids may also be formed from the β-phenylethyl amines. For example, Rc-CH_2-CH_2NH_2 and Rc-CH_2-CHO (from dopa) could combine to give nor-laudanosine (XLVI). Robinson (5) and Manske (112) have elaborated on these views. Robinson (5) has extended the theory to explain the formation of morphine (XLVII) from tyrosine. Recent tracer experiments by Battersby & Harper (113) and by Leete (114) support this view. Tyrosine-α-C^{14} was administered to *Papaver somniferum* and the morphine isolated and degraded chemically. Approximately half of the C^{14} in the morphine molecule was concentrated in position 16 (see XLVII) as predicted by Robinson's theory. The remainder of the C^{14} should be in position 9, but this has not been proven yet. Phenylalanine was also converted to morphine but was only one-fifth as efficient a precursor as was tyrosine (114).

Summary and Discussion

A number of routes exist for formation of benzenoid compounds from carbohydrates but so far only the shikimic acid and acetate pathways have been demonstrated in higher plants. The acetate pathway has been shown to operate for synthesis of ring A of flavonoids and probably some other rings bearing hydroxyls *meta* to each other are also formed from acetate. The shikimic acid pathway is most likely responsible for formation of a wide variety of secondary growth substances.

Some ramifications of the shikimic acid pathway are indicated in Figure 11. This scheme summarizes most of the work reviewed above. It is assumed that shikimic acid is converted to prephenic acid which then may

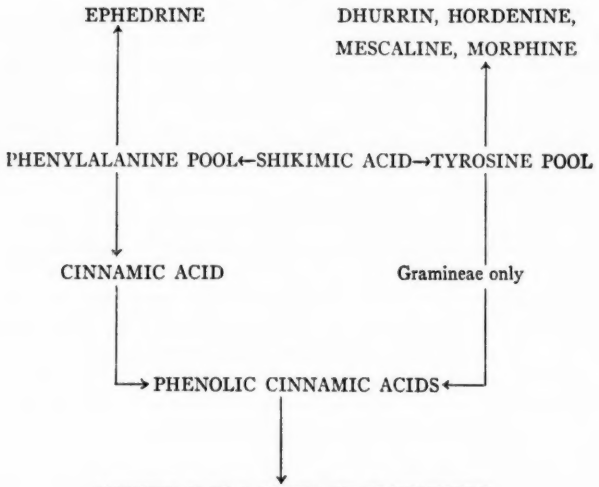


FIG. 11. Formation of some secondary growth substances by the shikimic acid pathway.

form either phenylpyruvic acid or *p*-hydroxyphenylpyruvic acid (Fig. 2), thus generating the phenylalanine and tyrosine pools respectively. These pools each contain the corresponding α-amino, α-keto, and α-hydroxy acids in equilibrium with one another. There is no reason to suppose a compound can pass from the tyrosine pool to the phenylalanine pool. Apparently some *p*-hydroxylation of the compounds occurs in the phenylalanine pool but this is probably not the main source of the intermediates in the tyrosine pool; rather they are likely to come directly from prephenic acid.

The intermediates of the phenylalanine and tyrosine pools serve as precursors of a wide variety of aromatic compounds. Decarboxylation of the amino acids may form intermediates which give rise to benzenoid nitrogen compounds such as certain alkaloids and cyanogenic glucosides. Dehydration of the hydroxy acids (or an equivalent reaction) forms cinnamic acid or *p*-coumaric acid and leads to formation of other phenolic cinnamic acids as shown in Figure 7. Not all species can convert the tyrosine pool intermediates to the phenolic cinnamic acids. To date this ability has been demonstrated only for some members of the family Gramineae. It is believed, however, that all species can convert the intermediates of the phenylalanine pool to cinnamic acid. Hydroxylation of cinnamic acid then leads to formation of the phenolic cinnamic acids. Reduction of these acids to the cinnamyl alcohols, followed by oxidative polymerization of the alcohols, probably plays a major role in formation of lignins. It is also likely

that a tryptophan pool is formed via shikimic acid (6, 7) and that it gives rise to other indole compounds.

During the course of evolution a relatively small number of qualitative changes in enzyme constitution might have resulted in production of the wide array of secondary growth substances. Certain enzymes of carbohydrate metabolism are not absolutely specific. Thus, Meister (115) has shown that muscle lactic acid dehydrogenase will reduce both phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid to the corresponding hydroxy acids. SentheShanmuganathan & Elsden (116) in a study of tyrosol formation by fermenting yeast have adduced evidence supporting the sequence: $Rp\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH \longrightarrow Rp\text{-}CH_2\text{-}CO\text{-}COOH \longrightarrow Rp\text{-}CH_2\text{-}CHO \longrightarrow Rp\text{-}CH_2\text{-}CH_2OH$. The last two steps are believed to be catalysed by the same enzymes that convert pyruvate to ethanol. As the ability to form some secondary growth substance developed, it is likely that mutations producing new enzymes have arisen but some of the steps responsible for formation of the end-product might be carried on by enzymes already present for other, more vital purposes. Enzyme studies are badly needed in order to obtain further information on biosynthetic pathways in higher plants. These should involve a search for new enzymes and perhaps also a closer examination of the specificity of some familiar enzymes concerned with the metabolism of amino acids, fats, and carbohydrates.

LITERATURE CITED

1. "The Metabolism of Secondary Plant Products," *Encyclopedia of Plant Physiology*, **10** (Rhuland, W., Ed., Springer-Verlag, Berlin, Germany, 1958)
2. Geissman, T. A., and Hinreiner, E., *Botan. Rev.*, **18**, 77 (1952)
3. *The Alkaloids*, vols. **1-5** (Manske, R. H. F., and Holmes, H. L., Eds., Academic Press, Inc., New York, N.Y., 1953)
4. Fraenkel, G. S., *Science*, **129**, 1466 (1959)
5. Robinson, R., *The Structural Relations of Natural Products* (Clarendon Press, Oxford, England, 1955)
6. Davis, B. D., *Advances in Enzymol.*, **16**, 247 (1955)
7. Davis, B. D., *Arch. Biochem. Biophys.*, **78**, 497 (1958)
8. Srinivasan, P. R., Shigeura, H. T., Sprecher, M., Sprinson, D. B., and Davis, B. D., *J. Biol. Chem.*, **220**, 477 (1955)
9. Srinivasan, P. R., Katagiri, M., and Sprinson, D. B., *J. Biol. Chem.*, **234**, 713 (1959)
10. Srinivasan, P. R., and Sprinson, D. B., *J. Biol. Chem.*, **234**, 716 (1959)
11. Weissbach, A., and Hurwitz, J., *J. Biol. Chem.*, **234**, 705 (1959)
12. Hurwitz, J., and Weissbach, A., *J. Biol. Chem.*, **234**, 710 (1959)
13. Ghosh, J. J., *Federation Proc.*, **15**, 261 (1956)
14. Schwink, I., and Adams, E., *Federation Proc.*, **17**, 308 (1958)
15. Tatum, E. L., Gross, S. R., Ehrensvärd, G., and Garnjobsts, L., *Proc. Natl. Acad. Sci. U.S.*, **40**, 271 (1954)
16. Metzenberg, R. L., and Mitchell, H. K., *Biochem. J. (London)*, **68**, 168 (1958)
17. Gross, S. R., *J. Biol. Chem.*, **233**, 1147 (1958)
18. Bassett, E. W., and Tanenbaum, S. W., *Biochim. et Biophys. Acta*, **28**, 247 (1958)

19. Higuchi, T., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958,* **2,** 161 (1958)
20. Mitsuhashi, S., and Davis, B. D., *Biochim. et Biophys. Acta,* **15,** 54 (1954)
21. Brown, S. A., and Neish, A. C., *Nature,* **175,** 688 (1955)
22. Eberhardt, G., and Schubert, W. J., *J. Am. Chem. Soc.,* **78,** 2835 (1956)
23. McCalla, D. R., and Neish, A. C., *Can. J. Biochem. and Physiol.,* **37,** 537 (1959)
24. Underhill, E. W., Watkin, J. E., and Neish, A. C., *Can. J. Biochem. and Physiol.,* **35,** 219 (1957)
25. Neish, A. C., *Can. J. Botany,* **37,** 1085 (1959)
26. Kosuge, T., and Conn, E. E., *Federation Proc.,* **18,** 265 (1959)
27. Kosuge, T., and Conn, E. E., *J. Biol. Chem.,* **234,** 2133 (1959)
28. Brown, S. A., Towers, G. H. N., and Wright, D., *Can. J. Biochem. and Physiol.,* **38** (1960)
29. McCalla, D. R., and Neish, A. C., *Can. J. Biochem. and Physiol.,* **37,** 531 (1959)
30. Gamborg, O. L., and Neish, A. C., *Can. J. Biochem. and Physiol.,* **37,** 1277 (1959)
31. Weinstein, L. H., Porter, C. A., and Laurencot, H. J., *Nature,* **183,** 326 (1959)
32. Gunsalus, I. C., Horecker, B. L., and Wood, W. A., *Bacteriol. Rev.,* **19,** 79 (1955)
33. Srere, P. A., Cooper, J. R., Klybas, V., and Racker, E., *Arch. Biochem. Biophys.,* 59, 535 (1955)
34. Calvin, M., *J. Chem. Soc.,* 1895 (1956)
35. Vishniac, W., *Ann. Rev. Plant Physiol.,* **6,** 115 (1955)
36. Moses, V., and Calvin, M., *Arch. Biochem. Biophys.,* **78,** 598 (1958)
37. Collie, J. N., *J. Chem. Soc.,* **63,** 329 (1893)
38. Collie, J. N., *J. Chem. Soc.,* **91,** 1806 (1907)
39. Birch, A. J., *Progr. in Chem. of Org. Nat. Prod.,* **14,** 186 (1957)
40. Erdtman, H., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958,* **2,** 1 (1958)
41. Birch, A. J., Massy-Westrop, R. A., and Moye, C. J., *Australian J. Chem.,* **8,** 539 (1955)
42. Watkin, J. E., Underhill, E. W., and Neish, A. C., *Can. J. Biochem. and Physiol.,* **35,** 229 (1957)
43. Geissman, T. A., and Swain, T., *Chem. & Ind. (London),* 984 (1957)
44. Shibata, S., and Yamazaki, M., *Pharm. Bull. (Tokyo),* **5,** 501 (1957); *Chem. Abstr.,* **52,** 13882 (1958)
45. Grisebach, H., *Z. Naturforsch.,* **12b,** 227 (1957)
46. Hutchinson, A., Taper, C. D., and Towers, G. H. N., *Can. J. Biochem. and Physiol.,* **37,** 901 (1959)
47. Goodwin, T. W., and Treble, D. H., *Biochem. J. (London),* **70,** 14 pp. (1958)
48. Weygand, F., Brucker, W., Grisebach, H., and Schulze, E., *Z. Naturforsch.,* **12b,** 222 (1957)
49. Blackwood, A. C., and Neish, A. C., *Can. J. Microbiol.,* **3,** 165 (1957)
50. Mitoma, C., Posner, H. S., and Leonard, F., *Biochim. et Biophys. Acta,* **27,** 156 (1958)
51. Ayengar, P. K., Hayaishi, O., Nakajima, M., and Tomida, I., *Biochim. et Biophys. Acta,* **33,** 111 (1959)
52. Higuchi, T., *Physiol. Plantarum,* **10,** 633 (1957)
53. Bruns, F. H., and Fiedler, L., *Nature,* **181,** 1533 (1958)

54. Hibbert, H., *Ann. Rev. Biochem.*, **11**, 183 (1942)
55. Bate-Smith, E. C., *Sci. Proc. Roy. Dublin Soc.*, **27**, 165 (1956)
56. Kremers, R. E., *Tappi*, **40**, 262 (1957)
57. Reznik, H., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 70 (1958)
58. Brown, S. A., Wright, D., and Neish, A. C., *Can. J. Biochem. and Physiol.*, **37**, 25 (1959)
59. Brown, S. A., and Neish, A. C., *Can. J. Biochem. and Physiol.*, **33**, 948 (1955)
60. Brown, S. A., and Neish, A. C., *Can. J. Biochem. and Physiol*, **34**, 769 (1956)
61. Wright, D., Brown, S. A., and Neish, A. C., *Can. J. Biochem. and Physiol.*, **36**, 1037 (1958)
62. Neish, A. C., *Can. J. Biochem. and Physiol.*, **35**, 161 (1957)
63. Neish, A. C., *Can. J. Botany*, **36**, 649 (1958)
64. Acerbo, S. N., Schubert, W. J., and Nord, F. F., *J. Am. Chem. Soc.*, **80**, 1990 (1958)
65 Nord, F. F., and Schubert, W. J., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 189 (1958)
66. Billek, G., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 207 (1958)
67. Byerrum, R. U., Flokstra, J. H., Dewey, L. J., and Ball, C. D., *J. Biol. Chem.*, **210**, 633 (1954)
68. Reid, W. W., *Chem. & Ind. (London)*, 1439 (1958)
69. Reznik, H., and Urban, R., *Naturwissenschaften*, **44**, 13 (1957)
70. Shimazono, H., and Nord, F. F., *Arch. Biochem. Biophys.*, **78**, 263 (1958)
71. Axelrod, J., and Szara, S., *Biochim. et Biophys. Acta*, **30**, 188 (1958)
72. Adler, E., *Ind. Eng. Chem.*, **49**, 1377 (1957)
73. Nord, F. F., and DeStevens, G., *Encyclopedia of Plant Physiology*, **10**, 389 (Springer-Verlag, Berlin, Germany, 1958)
74. Freudenberg, K., *Nature*, **183**, 1152 (1959)
75. Kratzl, K., and Billek, G., *Tappi*, **40**, 269 (1957)
76. Kremers, R. E., *Ann. Rev. Plant Physiol.*, **10**, 185 (1959)
77. Wardrop, A. B., and Bland, D. E., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 92 (1958)
78. Siegel, S. M., *Quart. Rev. Biol.*, **31**, 1 (1956)
79. Manskaja, S. M., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 215 (1958)
80. Kratzl, K., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 247 (1958)
81. Brown, S. A., and Neish, A. C., *J. Am. Chem. Soc.*, **81**, 2419 (1959)
82. Freudenberg, K., and Niedercorn, F., *Chem. Ber.*, **91**, 591 (1958)
83. Freudenberg, K., *Angew. Chem.*, **68**, 508 (1956)
84. Kratzl, K., and Hofbauer, G., *Monatsh. Chem.*, **87**, 617 (1956)
85. Kratzl, K., and Hofbauer, G., *Monatsh. Chem.*, **89**, 96 (1958)
86. Kratzl, K., Billek, G., Klein, E., and Buchtela, K., *Monatsh. Chem.*, **88**, 721 (1957)
87. Kratzl, K., and Faigle, H., *Monatsh. Chem.*, **89**, 708 (1958)
88. Freudenberg, K., *Angew. Chem.*, **68**, 84 (1956)
89. Adler, E., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958*, **2**, 137 (1958)
90. Higuchi, T., *Physiol. Plantarum*, **10**, 621 (1957)

91. Erdtman, H., *Modern Methods of Plant Analysis,* **3,** 428 (Paech, K., and Tracey, M. V., Eds., Springer-Verlag, Berlin, Germany, 1955)
92. Kratzl, K., and Buchtela, K., *Monatsh. Chem.,* **90,** 1 (1958)
93. Stone, J. E., Blundell, M. J., and Tanner, K. G., *Can. J. Chem.,* **29,** 734 (1951)
94. Bogorad, L., *Ann. Rev. Plant Physiol.,* **9,** 417 (1958)
95. Grisebach, H., *Z. Naturforsch.,* **13b,** 335 (1958)
96. Grisebach, H., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958,* **2,** 56 (1958)
97. Comte, P., Ville, A., Zwingelstein, G., Favre-Bonin, J., and Mentzer, C., *Bull. soc. chim. biol.,* **40,** 1117 (1958)
98. Zaprometov, M. N., *Fiziol. Rastenii, Akad Nauk S.S.S.R.,* **5,** 51 (1958); A.I.B.S. English transl., p. 46
99. Kursanov, A. L., and Zaprometov, M. N., *Atompraxis,* **4,** 280 (1958)
100. Watkin, J. E., and Neish, A. C., *Can. J. Biochem. and Physiol.* (In preparation)
101. Marion, L., *Bull. soc. chim. France,* **109** (1958)
102. Brady, L. R., and Tyler, V. E., *Plant Physiol.,* **33,** 334 (1958)
103. Lette, E., *Chem. & Ind. (London),* 604 (1959)
104. Conn, E. E., and Akazawa, T., *Federation Proc.,* **17,** 205 (1958)
105. Gander, J. E., *Federation Proc.,* **17,** 226 (1958)
106. Gander, J. E., *Federation Proc.,* **18,** 232 (1959)
107. Shibata, S., and Imaseki, I., *Pharm. Bull. (Tokyo),* **4,** 277 (1956); *Chem. Abstr.,* **51,** 7509 (1957)
108. Shibata, S., Imaseki, I., and Yamazaki, M., *Pharm. Bull. (Tokyo),* **5,** 71 (1957); *Chem. Abstr.,* **51,** 16734 (1957)
109. Imaseki, I., *Pharm. Bull. (Tokyo),* **5,** 594 (1957); *Chem. Abstr.,* **52,** 8286 (1958)
110. Imaseki, I., Shibata, S., and Yamazaki, M., *Chem. & Ind. (London),* 1625 (1958)
111. Leete, E., *Chem. & Ind. (London),* 1088 (1958)
112. Manske, R. H. F., *The Alkaloids,* **4,** Chap. 25, (Manske, R. H. F. and Holmes, H. L., Eds., Academic Press Inc., New York, N.Y., 1953)
113. Battersby, A. R., and Harper, B. J. T., *Chem. & Ind. (London),* 364 (1958)
114. Leete, E., *Chem. & Ind. (London),* 977 (1958)
115. Meister, A., *J. Biol. Chem.,* **184,** 117 (1950)
116. SentheShanmuganathan, S., and Elsden, S. R., *Biochem. J. (London),* **69,** 210 (1958)

CRASSULACEAN ACID METABOLISM[1,2]

By S. L. Ranson and M. Thomas
Department of Botany, King's College,
Newcastle upon Tyne, in the University of Durham, England

Under natural conditions the acid content of some nonhalophytic succulent plants increases at night and decreases on the following day (9, 13, 29, 85, 88, 113, 114). This type of diurnal variation in acidity was first discovered in the Crassulacean plant now known as *Bryophyllum calycinum,* and, in consequence, has been called Crassulacean Acid Metabolism (CAM).

During dark acidification in CAM (111) oxygen is continuously absorbed and, in the early stages, little or no CO_2 is evolved from the organ (i.e., its CO_2-output is low or zero); consequently the value of the measured RQ (respiration quotient) is low or even zero. Later CO_2 is evolved at an ever increasing rate and, at the completion of dark acidification, the value of the RQ reaches unity. During light deacidification in air (9, 89) oxygen is continuously evolved, but the absorption of atmospheric CO_2 by the illuminated green organs is sluggish. Consequently the measured photosynthetic quotient, i.e., the volume of oxygen evolved relative to the volume of CO_2 absorbed, has values greater than unity. While the light deacidification goes to completion there is an increase in the amount of CO_2 absorption, and the values of photosynthetic quotients fall to unity.

CAM occurs only in cells containing chloroplasts. The earlier experiments were carried out on whole plants under natural conditions, but the phenomenon can be studied in detached green organs kept in the laboratory under imposed conditions of alternating darkness and light. Pucher *et al.* (67) reported that quantitative changes in organic compounds were similar in attached and excised leaves of *B. calycinum* in the light and the dark. More recently (21, 22, 81, 116), changes in acidity and gaseous exchanges have been measured in discs and sections cut from green organs.

As regards the connection between succulence and CAM, the present position seems to be much the same as it was when Evans (29) reviewed the subject. It is doubtful whether this form of metabolism is shown by plants that do not possess some degree of succulence (but see 9). It is not, however, invariably present in succulents; and, even those succulents which possess it can survive without exercising it continuously to more than a minor degree (see p. 87). When it occurs, the pH of plant sap fluctuates (62, 69), the osmotic pressure may change (62), and the organic solids may

[1] The survey of literature pertaining to this review was concluded in August 1959.

[2] The following abbreviations are used: CAM (Crassulacean acid metabolism); DPN (diphosphopyridine nucleotide); $DPNH_2$ (reduced diphosphopyridine nucleotide); FW (fresh weight); PEP (phosphoenolpyruvate); PGA (phosphoglyceric acid); RDP (ribulose diphosphate); RQ (respiration quotient); TCA (tricarboxylic acid); $TPNH_2$ (reduced triphosphopyridine nucleotide).

increase in the dark as well as in light (67, 100). At least the last consequence would appear to be advantageous.

The extensive analyses by Pucher and Vickery, and their co-workers (64, 65, 67, 68, 69) of acids in attached and detached leaves of *B. calycinum,* together with the work of Wood (115) on *Sedum praealtum,* Moyse *et al.* (54) and Bruinsma (22) on *Bryophyllum* spp., and Bradbeer (16) on *Kalanchoë crenata,* all lead to the conclusion that the characteristic diurnal fluctuation in acidity in the Crassulaceae is predominantly owing to malic acid changes. In the dark, malate is produced in the cytoplasm and accumulates in the vacuole (p. 85). In the light, vacuolar stores of malate are depleted at a rate dependent on the light intensity as well as on the temperature. Presumably the acid diffuses from the vacuole to centres of metabolic consumption in the cytoplasm. Broadly, therefore, in the dark, acidification occurs because malic acid is synthesized and then removed to the vacuole faster than it is metabolically consumed via the TCA (tricarboxylic acid) cycle and other possible pathways. Accumulation stops in the light presumably because synthesis is slower than metabolic consumption.

Isocitric acid, often the acid present in highest concentration, and citric acid have behaved more variably than malic acid in different plants, and also in the same plant, under different physiological conditions. In *B. calycinum,* at normal temperatures, citrate has generally varied in phase with malate, but with a markedly smaller amplitude. Isocitrate has shown very little diurnal change (97) but, under prolonged periods in the light or dark, variations in level have been reported (98, 102, 111). A residual acid fraction that contained some oxalate has not varied appreciably. At reduced temperatures (68) there has been more variation in citrate and isocitrate but acidification has still been mainly attributable to malate. The behaviour of citrate and isocitrate in *S. praealtum* was essentially similar to that in *B. calycinum;* but in leaves of *B. daigremontianum* and *K. crenata* isocitrate occasionally varied more than citrate.

Because citrate, isocitrate, and other acids have behaved erratically, in an arbitrary abstraction of CAM from the total metabolism of green cells, we shall focus attention primarily on those linked reactions that lead to the accumulation of malate in the dark and to its disappearance in the light.

The central questions are these: Where do the four atoms in the malate synthesized in the dark come from? Where do they go in the light?

The long-known fact that the amount of dark acidification relates to the intensity of photosynthesis during the preceding light period pointed to carbohydrate as the source of acid. Large amounts of starch are consumed when leaves of *Bryophyllum* spp. (66, 69, 99, 111), *Sedum praealtum* (115), and *Kalanchoë crenata* (16) undergo dark acidification. As starch provides carbon for so many metabolic products it is not surprising that more than enough is often consumed overnight to account for the amount of acid that accumulates (p. 94). At the end of a night, indeed of a more prolonged dark period, there may be a considerable residue of starch left in the

leaves. Metabolic traffic in sedoheptulose has been reported but the accumulation of this substance in the light and disappearance in the dark is not invariably associated with CAM. The favoured view at present, therefore, is that insofar as the carbon skeleton of accumulating malate comes from an internal source, it comes from starch by way of hexose compounds. The mode of catabolism of hexoses during dark acidification is therefore a central problem in the study of CAM. Of particular current interest is the determination of the extent to which phosphoglyceric acid, and therefore phosphoenolpyruvic acid and, possibly, pyruvic acid, are formed by the glycolytic and pentose phosphate pathways respectively (see p. 102).

The discovery by Wood and Werkman of CO_2-fixation in the synthesis of dicarboxylic acids and the demonstration later of CO_2-fixation in animal tissues were pointers to investigations on CAM (14, 87). Troughs in the CO_2-output curve during dark acidification (9, 86, 110, 112) and consequent low values of RQs were regarded as possible fingerprints of dark CO_2-fixation (14, 87, 88, 113). For the over-all reactions Thomas postulated that oxygen absorption related exclusively to the complete oxidative catabolism of carbohydrate (Eq. 1), and that the CO_2 produced in this reaction is wholly or in part metabolically consumed according to over-all Equation 2, and only the unconsumed fraction is evolved. It will save space later if we describe these postulates as the Newcastle over-all hypothesis.

$$C_6H_{12}O_6 + 6O_2 = + 6H_2O \qquad 1.$$

$$C_6H_{12}O_6 + 2CO_2 = 2C_4H_6O_5 \qquad 2.$$

These over-all equations permitted all apparent RQ values (the resultants of the two processes depicted) from zero to unity to be accounted for in a CO_2-free atmosphere. It followed logically that, in a CO_2-enriched atmosphere, if the CO_2-fixation system of Equation 2 was not saturated by the CO_2 produced by Equation 1, additional CO_2 would be absorbed from the environment, i.e., there would be a negative CO_2-output and, therefore, a negative RQ. Massive dark fixation of CO_2 by leaves of *Bryophyllum* and *Kalanchoë* under these conditions was demonstrated (90) to be more than enough to account by Equation 2 for the measured accumulation of titratable acid (see p. 93). This massive dark fixation of CO_2 has been confirmed (31, 50, 92, 115, and see p. 87) for a number of succulent and semisucculent plants showing CAM. Moreover, under varying conditions of temperature, CO_2-concentration, etc., the course of acidification over a period runs roughly parallel to that of CO_2 fixation (92).

Shortly after Bonner & Bonner (14) had inferred CO_2-fixation in CAM from their observation that CO_2 feeding enhances dark acidification in *Bryophyllum* spp., Thurlow & Bonner (93) used $^{14}CO_2$ to establish conclusively that this inference was justified (see p. 100). The extent to which CO_2-fixation contributes in the synthesis of malic acid was not determined

by these or more recent experiments with $^{14}CO_2$. The reasonably close approximation (p. 93) of the quotient, acid-gain/CO_2-fixed, to the theoretical value required by Equation 2 still provides the best evidence that CO_2-fixation is involved in the production of most if not all of the malate which accumulates in CAM. Supporting evidence is provided by some of the values for the quotient starch-loss/acid-gain (see p. 95).

In those green organs, therefore, which are capable of showing CAM, i.e., massive dark accumulation of malic acid and dark CO_2-fixation at least equivalent to that required by Equation 2, there must be one or more extremely active carboxylating enzymes. These enzymes must govern the eventual dark incorporation of carbon coming from CO_2 produced by the oxidative catabolism of carbohydrate (Eq. 1) and, often, in addition, CO_2 absorbed from the environment, into malate molecules. Details of enzymic systems and other requirements for CAM are discussed on page 102.

If an organ showing CAM is maintained in the dark after it has accumulated acid to its maximum value, sooner or later dark deacidification sets in, and the RQ often rises well above 1.33 (9, 88, 89, 111). We do not know what causes dark acidification to cease or dark deacidification to begin. What is clear is that in the green organs there are enzymes concerned with the consumption of malate, and moreover, the rise in the RQ suggests that consumption involves decarboxylation. These observations define the problem whether or not the same carboxylases are concerned with malate synthesis and malic breakdown (see p. 96). Malate may undergo complete oxidative catabolism (Eq. 3), with an RQ of 1.3,

$$C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O \qquad 3.$$

but evidence has been reported (67, 98, 115) of starch synthesis during dark acidification. This evidence provides some support (but see p. 105) for the view put forward by Bennet-Clark (9) that those succulent plants that eventually show high RQ values in the dark may possess the power of converting malate into carbohydrate (Eq. 4). Evidently Equations 1 and 4 could give resultant RQ values greater than 1.33.

$$2\,C_4H_6O_5 = C_6H_{12}\,O_6 + 2CO_2 \qquad 4.$$

A general theoretical picture may be helpful. In the cytoplasm of green cells showing CAM, during dark acidification malate production (Eq. 2) occurs faster than malate consumption (Eqs. 3 and 4). At maximum acidification, production and consumption are balanced; during dark deacidification, consumption is faster than production. These ideas concerning dark deacidification have a bearing on the mechanism of light deacidification. Thomas (87, 88, 89) postulated that, under natural conditions, any CO_2 coming from intracellular sources or the environment is preferentially used in normal photosynthesis by illuminated green cells. Owing to a shortage or complete failure of the CO_2 supply at carboxylation centres (for more recent views see p. 104) in the cytoplasm, malate synthesis would be strongly

retarded or would stop when green organs are transferred from the dark to strong light. Malate consumption would continue however, and conceivably, under the control of those enzymes which bring about dark deacidification, possibly by the reactions shown in Equations 3 and 4. Such carbon dioxide as is produced in these reactions would be used in photosynthesis, thus sparing atmospheric CO_2 and, consequently, the apparent photosynthetic quotient would be greater than unity.

That the carboxylating system which causes malate synthesis in the dark still remains intact in the light has been proved by demonstrating massive light acidification in *Bryophyllum* and *Kalanchoë* leaves when, in a deacidified state, they were supplied with CO_2 in a concentration greater than that which saturates the photosynthetic mechanism (21, 51, 89, 90). This supports the view that it is a lack of CO_2 at cytoplasmic centres of acid synthesis, and not a change in intrinsic enzymic activity, that leads to light deacidification. Indeed, in the explanation of the diurnal variation of CAM, we have stressed the importance of the power possessed by the green organs (but only by green organs) of some succulent and semisucculent plants (when in the appropriate physiological state of having a high carbohydrate and a low initial content of malate) to fix CO_2 in malate synthesis, either in the dark or in the light. For unknown reasons the synthesized malate then accumulates in the vacuole. When the green organs are in this state, and certain other conditions [e.g., an oxygen requirement (see p. 90)] are satisfied, whether or not malate accumulates in them will be determined by the availability of CO_2 at the synthesizing centres. Under natural conditions, the CO_2 produced in oxidative catabolism is available for malate synthesis during the night but not during the day. Consequently, malate will be synthesized and will accumulate at night but not in daytime.

Details concerning the control of light deacidification are less clear. It is a much faster process than dark deacidification. Possibly this is not entirely the result of the continued production of malate in the dark, but not in the light, simultaneously with consumption. For example, light energy might actually enhance the oxidative consumption of acids (p. 106) or facilitate the movement of malate molecules from the vacuole to enzymic centres of consumption in the cytoplasm. These centres are not necessarily identical with those for malate production. What is clear is that the decarboxylative power displayed by the green organs in the light matches the high carboxylative power in the dark that characterizes CAM. These powers must be genetically conferred on the green organs by enzymic conjunctions and organizational properties which are peculiar to certain succulents and semisucculents.

CAM Power in the Dark

The course of dark acidification and the accompanying CO_2-fixation:— In normal CAM in whole leaves, dark acidification appears to set in at its maximum rate and the progress of acid accumulation (e.g., 92, 99, 111) and

the malic fraction (e.g., 99) appear to follow what might be logarithmic curves. The course of accumulation is usually [but see Bruinsma (22) and Zabka *et al.*, (116)] similar in leaf discs or sections (21, 81). At temperatures near 20°C., acidification is complete in about 12 to 20 hr. At lower temperatures the initial rate is lower but acidification continues for longer periods, and it has been confirmed (92, 99) that total acidification may be considerably greater than at higher temperatures. Explanations of this phenomenon continue to be tentative.

Such measurements of CO_2-fixation and acidification as have been made concurrently (50, 92, 115) indicate that these events progress in parallel, thus permitting inferences to be made about acidification from measurements of CO_2-fixation or vice versa.

In two varieties of *Kalanchoë blossfeldiana,* namely Tom Thumb and Feuer Blüte, neither of which will flower until subjected to induction periods of short days, the onset of dark CO_2-fixation shows contrasting behaviour. In Tom Thumb (see p. 88) CAM does not develop in plants kept in long days, but does not develop concurrently with ability to flower in plants transferred to short days. Gregory *et al.* (31) found that, in marked contrast to green organs of other plants showing CAM, the rate of dark CO_2-fixation increased to a maximum rate over a period of 12 hr. [Twelve hours, incidentally, was also the critical dark period for flower induction. This finding, which has been confirmed (78), suggested that there might be a causal connection between CO_2-fixation and the production of flowering hormone (see, however, 27).] Zabka *et al.* (116) have reported that in Feuer Blüte the power of CO_2-fixation leading to dark acidification is possessed by vegetative plants kept on long days, as well as by plants induced to flower by short-day treatment. Moreover in this variety, as is generally the case in CAM, dark acidification reached its maximum rate during the first 4 hr. after transfer from the light to the dark. A high rate was maintained thereafter for about 12 hr. but eventually CO_2-fixation and acidification fell to zero.

Maximum amounts of dark acidification and dark CO_2-fixation:—In one Connecticut experiment on *B. calycinum* carried out in air at room temperature (69) organic acid increased in the dark from about 2200 mg. per 100 gm. fresh weight (FW) of leaves to 4000 mg. The increase of about 1800 mg. was accounted for by 1500 mg. malic acid and 300 mg. citric acid. Isocitric acid was the main component of the acid fraction (2200 mg.) that did not fluctuate diurnally. In experiments performed in Europe which have given qualitatively similar results (e.g., 115) the maximum acidification has been less, not exceeding 1000 mg. as a rule. Acidification has been augmented by simultaneously lowering the temperature and raising the external CO_2 concentration (92). In Bombay, Bharucha & Joshi (12) found the maximum dark acidification of *B. calycinum,* which occurred in the winter season, was 2682 mg. per 100 gm. fresh weight leaves, the highest value yet recorded.

The corresponding values for total organic acid contents were not, apparently, recorded in these particular experiments carried out by the Bombay workers. The Connecticut value of approximately 4000 mg. per 100 gm. fresh weight of leaves, obtained by Pucher, Vickery, Abrahams, and Leavenworth (69), appears therefore to be the maximum total acidity (including isocitrate and other invariable acids) so far recorded for *B. calycinum*.

Direct measurements of dark CO_2-fixation by *K. crenata* have been made at Newcastle. The highest value obtained has been 290 mg. per 100 gm. FW leaves. In this experiment the leaves were strongly illuminated for a preliminary period and then placed at 10°C. in an atmosphere containing 5 per cent CO_2. On the basis of the theoretical quotient of 3.05 for acid-accumulation/CO_2-fixation, the maximum calculated dark CO_2-fixation in mg. per 100 gm. FW leaves would be about 900 mg. in Bombay, about 600 mg. in Connecticut, and about 400 mg. in Europe. If we took the average value of 2.6, found by experiments at Newcastle (see p. 93), for the quotient, the calculated CO_2-fixation figures would be higher. It is this massive CO_2-fixation in certain succulents and semisucculents which characterizes CAM (see p. 83).

Massive though maximum dark CO_2-fixation is, it is usually considerably less than maximum light CO_2-fixation in photosynthesis by plants showing CAM. For example, in parallel experiments at Newcastle on *Kalanchoë* leaves in a CO_2-enriched atmosphere, in 8 hr., 1142 mg. of external CO_2 were fixed per 100 gm. FW leaf in bright light and 220 mg. in the dark. There are, however, records (31, 40) of experiments on *Kalanchoë* spp. in which conditions were such that dark CO_2-fixation exceeded photosynthetic fixation of external CO_2: indeed CO_2 evolution was observed (31) immediately after transfer from dark to the light (see also p. 107). In Connecticut it has been observed (e.g., 100) that during the period of acidification, but not subsequently, organic solids may increase as rapidly as during the preceding light period.

Varying CAM power in a single plant:—Older work (9, 88, 114) on the effects of age and season has been confirmed. The power of leaves to show dark acidification or dark CO_2-fixation develops as they unfold, increases until they are fully expanded, and decreases as they become senescent (2). Possibly, therefore, the results of experiments performed on very young leaves are not comparable in all respects with those obtained from experiments on mature leaves (see p. 102). In *Sedum praealtum* diurnal fluctuation in acidity was clearly discernible and regular only in the summer (April to October) in England, i.e., when photosynthesis is active during the day. In 1933 Bennet-Clark (9) found maximum acidification in July; in 1947 Wood (115) observed it in September. She suggested that, "The high early morning acidities found in September are probably due to the low night temperatures during that month in 1947." Bharucha & Joshi (12) carried out seasonal experiments on leaves attached to plants of *B. calycinum*

growing under tropical conditions in Bombay. They recorded percentage increases in night acidification of 1137, 3084, and 3959 in the monsoon, summer, and winter conditions, respectively. In contrast to Europe there appears to be no close season for CAM in Bombay. The authors attributed the high value for winter to days of bright sunshine followed by cooler nights than in the summer.

In the close season at Newcastle we have augmented the powers of leaves of *K. crenata* to show CAM in the winter by keeping the plants for a preliminary period under artificial illumination for long days. This practice derives from the observation made by Thoday & Richards (86) that troughs in the curve for CO_2-output (see p. 83) for *Kleinia sp.* in the dark were no longer seen in November, but appeared again when the plants were given extended illumination for five days. Zabka *et al.* (116) have compared the powers of discs cut out from leaves of plants of *K. blossfeldiana* var. Feuer Blüte (see last section), grown in long and short days respectively, to incorporate $^{14}CO_2$ into organic acids in the dark. The discs from both sets of plants showed high powers from the beginning of the dark period. The rate of CO_2-fixation was faster in the long-day plants.

In contrast to this response was the extraordinary behaviour of *K. blossfeldiana* var. Tom Thumb which had been previously observed by Gregory *et al.* (31) and studied further by Spear & Thimann (78). Kept in long days these plants did not develop the capacity to fix CO_2 in the dark or to flower. These capacities developed, however, after subjecting plants to 13 or more short days. Dark CO_2-fixation increased tenfold to a maximum between the 13th and 28th day. Neyland & Thimann (56) showed later that dark acidification typical of CAM occurred in such plants. This development of CAM power was arrested when the plants were illuminated for ten minutes in the first half of the dark period. Since, therefore, in this variety the development of "CO_2 metabolism is strongly influenced by photoperiodic treatment" it would appear to be promising material for investigating the internal conditions that determine whether or not CAM will take place.

The results recorded by Gregory *et al.* (31) suggested that the Tom Thumb plants kept continuously in long days were not completely lacking in powers of dark CO_2-fixation. Using more delicate methods of measurement, Zabka *et al.* (116) found that such plants fixed CO_2 in the dark at a steady but slow rate. This rate was greatly augmented by short-day treatment over a prolonged preliminary period. That the effect of exposure to short days is quantitative and not qualitative was the conclusion drawn by Kunitake *et al.* (42). They treated leaves of Tom Thumb plants with $^{14}CO_2$ for 10 to 30 min. in the dark and found the pattern in the organic and amino acids synthesized to be the same in noninduced plants kept continuously in long days as that in induced plants that had received short days. The rate of dark fixation was, however, greatly increased by short-day treatment. If these facts add up to the conclusion that the whole cellular

machinery for CAM is there and operating before short-day treatment, the question would be what kind of deficiency in the activity of spare parts limits the activity of the whole machinery and how is this made good during a period of 13 to 28 short days? A similar question might be put for developing leaves and for the seasonal changes in temperate regions from virtually zero activity in the winter to maximum activity in the summer and, in Bombay, from low activity in the monsoon season to maximum activity in the winter. The machinery (see p. 102) has many enzymic and coenzymic spare parts which may be synthesized or undergo re-arrangement in cells, and there may be inhibitors that are produced or removed.

A beginning has been made on the difficult task of measuring changes in enzyme activity. The activities of malic enzyme, malic dehydrogenase (26), and PEP (phosphoenolpyruvate) carboxylase (81, 103) were not very different in extracts from *Bryophyllum* and *Kalanchoë* leaves made at times when the leaves showed marked powers for CAM and when they showed slight powers of dark CO_2 fixation. Qualitative work has proceeded further; it appears that all the enzymes considered up to the present date in connection with malate synthesis in CAM are widely distributed in plant tissues. Possible differences between dark CO_2-fixation in tissues that do and do not show CAM are discussed on p. 102.

Oxygen and Dark Acidification in CAM

The influence of different external oxygen concentrations:—It has often been affirmed [Moyse (50)] that an external oxygen supply is essential for the generation of acids in CAM, and that the amount of dark acidification relates to the external oxygen concentration. Moyse (50) measured dark acidification in leaves of *B. daigremontianum* in CO_2-free atmospheres containing oxygen concentrations over the range <0.1 per cent (i.e., the oxygen percentage in cylinder nitrogen) to 100 per cent, and when these atmospheres were enriched with 7 per cent CO_2. In CO_2-free atmospheres he found a remarkable increase in acid accumulation (and in the calculated accompanying fixation of endogenously produced CO_2) as the oxygen concentration was raised from the lowest value to 5 per cent. As it was in this range that the velocity of oxygen consumption increased most rapidly [see also (52)] he inferred that oxygen acts by promoting those cellular decarboxylations which provide the substrate CO_2 required for malate synthesis. In CO_2-free air, the limiting factor in malate synthesis is the CO_2 supply to centres of synthesis. Support for this view came from the results of his experiments with 7 per cent CO_2. There was no clear drift of acidification with oxygen concentrations increasing from 1 per cent. With ample external CO_2, the rates of intracellular oxidative decarboxylations were no longer limiting CO_2-fixation.

The possibility still remains that oxygen may function in other ways besides controlling rates of decarboxylation, for example, in the transfer-

ence of malate to the vacuole (see p. 85). Moyse pointed out that, if CO_2 supply were the only factor to be considered, dark acidification should occur in the absence of oxygen as long as anaerobic decarboxylations went on. None of his experiments appear to have been strictly anaerobic, but he concluded from those in which he used cylinder nitrogen that there is practically no anaerobic fixation of CO_2. The results obtained at Newcastle (2, 63) support this conclusion. For *Kalanchoë* leaves in pure nitrogen Avadhani (2) found that, although titratable and total acid varied slightly [cf. Moyse (50)], malic acid as determined by the specific *Lactobacillus* technique (Nossal (59)] showed no increase and possibly tended to decrease slightly over prolonged periods. Leaves exposed to $^{14}CO_2$ in nitrogen, either immediately or after a few hours (when, probably internal as well as external conditions were truly anaerobic), incorporated ^{14}C almost exclusively in malic acid, but always in such low amounts compared to aerobic incorporation as to leave doubts about a net fixation. The anaerobically labelled malate contained ^{14}C only in the carboxylic groups. After one hour, 80 per cent of the label was in the β-COOH group but after 12 hr. there was almost as much in the α- as in the β-COOH group. This is markedly different from the labelling pattern in air. Together these results suggest that (*a*) there is no appreciable accumulation of malate under completely anaerobic conditions, and (*b*) if any malate synthesis occurs it is of a very minor order and follows a somewhat different pathway from that under aerobic conditions. The incorporation of ^{14}C initially into the β-COOH group of the malate could well have occurred without net synthesis. The spread of label to the other carboxylic group suggests randomization by way of fumarase and fumaric acid.

Paxton (63) had shown earlier that malate synthesis soon stops and anaerobic zymasis occurs in purified nitrogen. In one experiment 40 mg. ethanol accumulated in 10 gm. FW leaves in 24 hr. Avadhani (2) detected a slight accumulation of succinate. Leaves are often visibly injured during anaerobiosis and, as Moyse (50) suggested, products of anaerobic catabolism might inhibit one or more of the reactions by which carbohydrate is converted into malate in CAM. By making measurements of acidification and CO_2-fixation before and after anaerobic periods with the appropriate controls, Avadhani (*loc. cit.*) obtained evidence that, over a limited anaerobic period, the power to synthesize malate with CO_2-fixation is not permanently impaired.

The influence of terminal oxidase inhibitors and of arsenite:—Somers (77) reported that cyanide strongly inhibited dark acidification. Since one of the CO_2-fixations in CAM (see p. 102) may resemble photosynthetic fixation by being very sensitive to cyanide, this inhibition does not prove that malic accumulation depends on the activity of terminal oxidases containing heavy metals. Brown (21) observed a depression of oxygen uptake of about 50 per cent in $5 \times 10^{-3}M$ cyanide, and dark acidification was

depressed to a maximum of 90 per cent. Malate accumulation was affected more than citrate accumulation. Whilst the controls were fixing CO_2 the cyanide-treated sections were evolving CO_2, and the RQs rose above unity. Ethanol and acetaldehyde, but not lactic acid, accumulated simultaneously, and in amounts which suggested the possibility that the catabolism of phosphoenolpyruvate might be diverted from the pattern which leads to malate synthesis to that which leads to cyanide-zymasis (91) via pyruvate. In fact, Brown detected the accumulation of pyruvate. When the cyanide was removed, fixation and dark acidification of CAM began again.

Brown (21) confirmed that azide inhibits dark acidification [Somers, (77)], and reported that azide also induced other changes, similar to those occasioned by cyanide and might, in addition, interfere with the production of the C_3 acceptor of CO_2.

Brown also found that the oxygen-uptake of leaf sections of *Kalanchoë* was very strongly retarded by $10^{-2}M$ arsenite (a TCA-cycle inhibitor) and that a little α-oxoglutarate accumulated. Dark acidification was strongly retarded, and marked arsenite-zymasis occurred with some pyruvate accumulation. The results with arsenite, therefore, resembled those with inhibitors of terminal oxidases in that they tended to show a connection between aerobic catabolism and dark acidification in CAM. The responses to arsenite are different (see p. 92) from those to malonate and fluoroacetate, two other inhibitors of TCA-cycle reactions. No reason for this can be suggested yet.

The fate of absorbed oxygen:—Leaves undergoing dark acidification in CAM behave like other leaves insofar as they show a continuous absorption of oxygen from the environment after transference from the light to the dark. In normal leaves amply stocked with carbohydrate, RQs approximate unity for many hours in the dark, indicating that the complete oxidative catabolism of carbohydrate (Eq. 1) is the dominant process involving oxygen uptake. The question of the fate of oxygen absorbed by leaves synthesizing malic acid in CAM is not so easily settled because RQs are less than unity and may be zero. As we have seen (p. 83) this could happen if Reaction (1) proceeded as in normal leaves, but some or all of the CO_2 produced was metabolically consumed in the synthesis of malate by the reaction in Equation 2. However, it is possible that some malate is produced, with oxygen uptake, from carbohydrate by way of the TCA cycle and accumulates in the vacuole. Such a net synthesis of malate implies a previous synthesis of oxaloacetate (with CO_2 fixation) to combine with acetyl coenzyme A, and thus to yield citrate. In outline the oxidative conversion of hexose to citrate and malate may be represented thus:

$$\text{HEXOSE} \xrightarrow{1\frac{1}{2}O_2} \begin{cases} \text{PEP (or pyruvate)} \\ CO_2 \\ \text{Acetyl} \end{cases} \begin{cases} \text{Oxaloacetate} \\ \downarrow \quad 1\frac{1}{2}O_2 \\ \text{Citrate} \longrightarrow \text{Malate} + 2CO_2 \end{cases}$$

The over-all equation for the production of malate by this pathway would be:

$$C_6H_{12}O_6 + 3O_2 = C_4H_6O_5 + 2CO_2 + 3H_2O. \quad 5.$$

The RQ for this conversion is 0.67. The over-all equation for the production of citrate would be:

$$2C_6H_{12}O_6 + 3O_2 = 2C_6H_8O_7 + 4H_2O. \quad 6.$$

As the RQ during the most intense phase of dark acidification in air is always well below 0.67, Reaction 5 cannot be the sole way of synthesizing malate. However, if the CO_2 produced by Reaction 5 were all consumed by Reaction 2 we get another over-all equation (Eq. 7) for malate production:

$$2\ C_6H_{12}O_6 + 3\ O_2 = 3\ C_4H_6O_5 + 3\ H_2O \quad 7.$$

which has a zero RQ value and therefore satisfies the requirements of experimental findings for CAM. Equation 7 also summarizes the linkage of Reactions 1 and 2 [Moyse (50)], i. e., the Newcastle over-all hypothesis.

The production of a considerable fraction of accumulating malate by aerobic oxidative processes might be reflected in a relation between the rates of oxygen uptake and acidification. Bennet-Clark (9), Wolf (111), and, more recently, Moyse (50), have reported instances of CAM in which such a relation has existed. However, there are results for entire leaves and discs or sections, obtained at Newcastle (21, 90, 92) and elsewhere (22, 81) which suggest that dark acidification in air, or in atmospheres enriched with CO_2, may be accompanied by a steady oxygen uptake. This is in accord with the Newcastle over-all hypothesis, namely that Reactions 1 and 2 are in dominant control of gaseous exchange during malate synthesis. Reaction 1 would continue as long as oxidizable carbohydrate remained in the leaves. It manifests itself clearly when the RQ reaches a value close to unity at the completion of dark acidification. Support for the idea of the persistence of a steady "respiration" during dark acidification comes from some of the records published by Gregory *et al.* (31) for CO_2-output before the slow beginning and after the completion of dark acidification in leaves of plants of *K. blossfeldiana* var. Tom Thumb which had been induced by short-day treatment to fix CO_2. Immediately after the lights were switched off, the rate of CO_2 output rose to a peak value. Possibly we may take this value as approaching the respiratory power of the leaves at the beginning of the dark period. The leaves were still kept in the dark after CO_2-fixation had run its course. Eventually a steady CO_2-output was observed, and at a rate near to that of the peak value reached at the beginning of the dark period.

Brown (21) studied the influence of certain inhibitors on dark acidification and the accompanying gaseous exchange of leaf sections, and obtained some support for the view that malic synthesis in CAM proceeds mainly by the carboxylative rather than the oxidative pathway. For example, he observed a 50 per cent reversible inhibition of oxygen uptake by tissue slices floated in buffer solution containing $10^{-2}M$ malonate at pH 4.0 in an at-

mosphere containing 0.1 per cent CO_2. This was accompanied by succinate accumulation and removal. In spite of the depressed oxygen uptake, malic acid, and to a lesser degree, tricarboxylic acids accumulated in the slices to at least as great an extent as in the control experiment. In fact, CO_2-fixation was much greater in the presence of malonate, indicating an augmented diversion of carbohydrate catabolism from the oxidative consumption of phosphoenolpyruvate to β-carboxylation. The results of Brown's experiments with $10^{-2}M$ sodium fluoroacetate would bear a similar interpretation. In these, not unexpectedly, the accumulation of tricarboxylic acids increased in the presence of the inhibitor.

Certain Quantitative Relations and the Newcastle Over-all Hypothesis

The quotient malic acid gain/CO_2 fixed:—If Equations 1 and 2 represent the only reactions involving gaseous exchange during dark acidification, this quotient may be calculated (90, 92) from simultaneous measurements of increase in titratable acid (assumed to be entirely malate), of oxygen-uptake, and of CO_2-uptake or CO_2-output. From Equation 2 the theoretical value of the quotient should be 3.05 by weight and unity by moles. The weight values for over a hundred Newcastle experiments (92) with *K. crenata* have ranged from a little below 2 to about 3.5, with a mean value about 2.6. For *S. praealtum,* Wood (115) obtained moles quotients varying between 0.54 and 1.9 with a mean value of 0.9. Moyse (50) used Equation 7, which combines Equations 1 and 2, to compare measured with theoretical values for *B. daigremontianum* in CO_2-free and CO_2-enriched atmospheres containing different amounts of oxygen. In low oxygen concentrations the measured values were anomalous, but in the range 10 to 20 per cent oxygen his results, like those reported above, indicated that the measured acid accumulation was less than that calculated by Equations 1 and 2 from measurements of gaseous exchange.

The possibility exists, therefore, that some of the CO_2 fixed in CAM becomes incorporated by side reactions in substances less acidic than malic acid (92). Lyndon (45) tested this possibility for nitrogenous compounds, but found no major accumulations of any nitrogen fractions during dark acidification. However, some evidence has been obtained that the increase in total acidity may be greater than the increase in titratable acidity (16, 39, 54, 81). Accordingly the possibility of neutralization of synthesized acids by basic substances in cells cannot be excluded yet. Moreover, the assumptions in the Newcastle over-all hypothesis may not be completely valid. Thus, Equation 1 may not represent the only reaction involving oxygen uptake. Conceivably, there is a net oxygen uptake in producing the CO_2 acceptors [now believed to be ribulose diphosphate and phosphoenolpyruvate (see p. 102)] in the conversion of hexose into malate. It would be unprofitable to discuss this idea until linkages of partial reactions that involve pyridine nucleotides have been elucidated (see p. 98). As yet, however, there appears

to be no reason to doubt (see p. 102) that for every molecule of hexose carbohydrate converted into two molecules of malate (Eq. 2) in CAM two molecules of CO_2 are metabolically fixed, whether or not this conversion involves a net oxygen uptake.

The quotient carbohydrate loss/acid gain:—Conceivably, some succulents and semisucculents might contain carbohydrate reserves other than starch from which malate might be derived in the required amounts during dark acidification. Evidently the over-all Equation 2 would have to be altered if a C_6 carbohydrate were not the main source of malate: for a C_7 carbohydrate reserve (e.g., sedoheptulose) the net fixation of CO_2 would be one molecule: for a C_5 carbohydrate reserve it would be three molecules. [The early work of Spoehr (79) on pentosans in succulents does not appear to have been developed in recent years.]

In the present section we shall consider the Newcastle over-all hypothesis in the light of quantitative measurements by masterly analytical methods which have been made at Connecticut of simultaneous starch consumption and dark acidification. Unfortunately we are left in doubt about the accompanying gaseous exchanges.

The easiest results to interpret would be from experiments done in CO_2-free atmospheres with measurements made over the period that the RQ remains at zero. Then from Equation 7 (which combines Equations 1 and 2 or 1 and 5) the quotient starch-loss/acid-gain would be 0.67 by moles, 0.89 by weight, or 1 by carbon units, if during the period starch were exclusively consumed according to Equations 1, 2, and possibly 5. Evidently, in an atmosphere containing CO_2, carbohydrate would be drawn on to a less extent for providing, by Reaction 1, the CO_2 required for Reaction 2. In the limiting case, in which the environment provided all the CO_2 fixed in Reaction 2, the quotient starch-loss/acid-gain would be 0.5 by moles, 0.67 by weight, and 0.75 by carbon units.

The Connecticut experiments on *B. calycinum* appear to have been carried out in ordinary air, i.e., under conditions in which the environment could provide some of the CO_2 fixed in malate synthesis. Vickery realized this and attributed to CO_2-fixation the increase in organic solids shown during the opening 12 to 16 hr. of darkness in some of his experiments [see also, earlier suggestions of Bennet-Clark, (8)]. In fact he expressed the view that the situation is complex in the early hours, as CO_2 is drawn from outside. Evidently in such experiments, if during the period of intense acidification the RQ values were negative or zero, the quotient starch-loss/acid-gain should lie between 0.67 and 0.5 by moles, between 0.89 and 0.67 by weight, and 1 and 0.75 by carbon units, if the sole fate of hexose were complete oxidation and conversion into malate. Higher values than the theoretical maxima, such as have often been obtained at Connecticut, and sometimes elsewhere (115), would suggest the possible consumption of hexose in additional ways. Unless citrate accumulation has been proved to accompany these higher values, we shall not consider them here.

If we have interpreted them correctly, some of the Connecticut data for starch loss and acid gain give quotients not widely different from those required by the Newcastle hypothesis. Thus data reported by Pucher *et al.* (69) give moles quotients of 0.93, 1.09, and 0.66 for three successive nights. Vickery (99) made measurements after 4, 8, and 24 hr. of darkness on a single night, and the successive quotients work out at 0.27, 0.5, and 1.1. The first value is only half the minimum theoretical value. Possibly soluble sugars may have been consumed as well as starch during this opening period.

Earlier, Vickery (97) measured starch loss and the malate component of net acid gain. For two successive 9 hr. periods in the dark with an intervening 16 hr. period in the light the starch-loss/malate-gain quotients for moles were 0.68 and 0.51, fitting the Newcastle hypothesis very well, if citrate did not accumulate appreciably. Later Vickery (99) measured citrate and malate accumulations separately in an experiment in which he described the citrate synthesis as "striking." At 24°C., after 8 hr. in the dark, the moles quotient for starch-loss/malate-gain was 1.06, but had risen to 1.8 by 16 hr. There was a definite increase in organic solids, i.e., CO_2 fixation took place. Consequently the theoretical quotient should have been less than 0.67. The inference is that some substance besides malate was formed from starch. Vickery reported that after 8 hr. the malate/citrate quotient was 2 and that it had risen to 2.5 after 16 hr. From these quotients and the molar relations of Equations 6 and 7 we can calculate, from Vickery's results for total starch loss and malic acid gain, the value of moles quotients for starch loss not attributable to citrate accumulation/malate-gain. These are 0.61 at 8 hr. and 1.12 at 16 hr. The second value is considerably higher than 0.67, but it is almost certain that, after 8 hr. in the dark, the leaves would be giving off CO_2. In parallel experiments at 6°C. the total starch-loss/malate-gain was 1.25 after 8 hr., and, we estimate from Vickery's charted data, the malate/citrate quotient was not greater than 3. The quotient starch loss not attributable to citrate accumulation/malate-gain works out at 0.83., i.e., distinctly higher than the theoretical quotient. We do not know, however, whether or not there was an accumulation of sugars in the leaves at this subnormal temperature.

Later, Vickery (101) again measured gains in citrate and malate as well as starch loss, but expressed his results in terms of carbon millimoles. In CO_2-free air, the theoretical quotient for starch-loss/acid-gain for each acid and therefore for the sum of the two, when expressed in carbon units, is unity (Eqs. 6 and 7). The data recorded by Vickery indicate that, after 12 and 18 hr. in the dark, the actual quotients were 0.68 and 0.96 respectively. In this experiment, as Vickery pointed out, more acid (malate plus citrate) accumulated during the opening period of 12 hr. than could be accounted for by carbohydrate loss, and he made the crucial point that this could be accounted for by fixation of atmospheric CO_2. From Equation 2 we see that, if the CO_2 fixed came entirely from the atmosphere, the quotient starch-loss/malate-gain would be 0.75 in carbon units. Clearly, therefore, the fix-

ation of atmospheric CO_2 in malate synthesis, quite apart from what may happen in citrate synthesis, would tend to reduce the quotient, starch-loss/total-acid-gain, well below unity.

In general, Vickery's data appear to us to provide supporting evidence for the view that oxygen uptake during dark acidification is mainly governed by Equation 1 (although they do not exclude the possible occurrence of Equation 5 also), and that when the RQ is zero (or negative) carbohydrate catabolism may result predominantly in the accumulation of malate, with the frequent accompanying accumulation of citrate. Results obtained at Newcastle by Bradbeer (16) led him to come to a similar conclusion.

Enzymes

The production and consumption of malic acid in CAM, by mechanisms visualized at present, would require the participation of enzymes of the glycolytic and pentose phosphate pathways of carbohydrate catabolism and of the TCA cycle in addition to one or more carboxylase systems. The occurrence of these in tissues showing CAM is discussed below.

Enzymes of the glycolytic and pentose phosphate pathways:—Direct demonstrations of the presence of these enzymes in leaves showing CAM are few. Phosphoglyceryl kinase is present in aerial parts of *Bryophyllum* (3); glucose-6-phosphate dehydrogenase (81), and 6-phosphogluconate dehydrogenase are present in *Kalanchoë* leaves. Indirect evidence is more abundant. The occurrence of zymasis in *Kalanchoë* leaves deprived of oxygen (p. 90) and in the presence of cyanide or arsenite (p. 91) suggests the operation of the glycolytic pathway. The production in *Kalanchoë* leaves of essentially the same labelled products as occur in other green tissues after short periods of photosynthesis in the presence of $^{14}CO_2$ (57, 58) argues for the presence of RDP (ribulose diphosphate) carboxylase and some at least of the enzymes of the glycolytic and pentose phosphate pathways.

Beevers & Gibbs (7, 30) who used the Bloom and Stetten technique of comparing $^{14}CO_2$ liberation from glucose-1-^{14}C and glucose-6-^{14}C to evaluate the relative contributions of the glycolytic and pentose phosphate pathways in glucose dissimilation in *Bryophyllum* obtained C-6/C-1 quotients of 0.75 for stem tissue and 0.54 for the leaves. Though doubts as to the general validity of the method have been expressed (4), and the results for *Bryophllum* may be further complicated by CO_2 fixation, the C-6/C-1 quotient may be taken to represent the maximum contribution of the glycolytic pathway. This would mean that the process in leaves involves about equal participation of the glycolytic and pentose phosphate sequences of reactions, whereas in the stem tissue the latter sequence is less important. Stiller's investigations (81) of the pathways operating in *Kalanchoë* leaves by the use of inhibitors were not conclusive, but her feeding experiments with labelled sugars and gluconate (p. 102) suggest the participation of the pentose phosphate sequence.

Carboxylases:—Enzymes that may be concerned in the synthesis of acids

in Crassulacean leaves, with CO_2-fixation, or, alternatively, in their consumption with CO_2 liberation, include malic enzyme, PEP carboxylase, PEP carboxykinase, and possibly, isocitric enzyme.

Malic enzyme (61) which catalyses the reaction shown in Equation 8 and also the decarboxylation of oxaloacetate is widely distributed in plant

$$\text{pyruvate} + CO_2 + TPNH_2 \rightleftharpoons \text{malate} + TPN \qquad 8.$$

tissues (96) including leaves which show CAM (1, 26). PEP carboxylase (Eq. 9), first described by Bandurski & Greiner (5, 6), is very widely distrib-

$$PEP + CO_2 \rightleftharpoons \text{oxaloacetate} + \text{inorganic phosphate} \qquad 9.$$

uted in green and nongreen plant tissues (35, 47) including Crassulacean leaves (74, 103, 104, 106). Reaction 9 coupled with Reaction 10 catalysed by malic dehydrogenase, which is very active in all tissues studied, would lead to the synthesis of malate from PEP and CO_2. PEP carboxykinase, which

$$\text{oxaloacetate} + DPNH_2 \rightleftharpoons \text{malate} + DPN \qquad 10.$$

according to Mazelis & Vennesland (47) is also very widely distributed in plants, catalyses reaction 11. This enzyme has not yet been reported for

$$PEP + CO_2 + ADP \rightleftharpoons \text{oxaloacetate} + ATP \qquad 11.$$

tissues showing CAM. Isocitric enzyme or isocitric dehydrogenase (49, 60, 95) which is widely distributed in plant tissues (96, 109) including leaves showing CAM (26, 103), catalyses Reaction 12.

$$\alpha\text{-oxoglutarate} + CO_2 + TPNH_2 \rightleftharpoons \text{isocitrate} + TPN \qquad 12.$$

At low CO_2 concentrations the equilibrium of the malic enzyme system (Eq. 8) in isolation is unfavourable to malate synthesis and it has been shown (33) that at pH 7 and 22°C., in the presence of 5 per cent CO_2 and a $TPNH_2/TPN$ quotient of unity, the malate/pyruvate quotient would be only 0.035. In mature *Kalanchoë* leaves the quotient is at least 100 (16), hence Walker (104) has stated that if malic acid accumulation in *Kalanchoë* is based upon malic enzyme there must be not only a mechanism for maintaining a high $TPNH_2/TPN$ quotient but another for removing malate from the site of synthesis at an extrememly rapid rate. The carboxylation of PEP to yield oxaloacetate by PEP carboxylase (Eq. 9) is virtually irreversible (84) and under natural conditions the conversion of oxaloacetate into malate by malic dehydrogenase (Eq. 10) is essentially complete. Walker concluded therefore that acidification by way of these reactions could occur at near maximal rates even if malate is not rapidly removed from the site of synthesis.

Stiller (81) has discussed the participation of these carboxylase systems in CAM, and points out that even if the $TPNH_2/TPN$ quotient in the malic enzyme system were maintained at 1000 the malate/pyruvate quotient would still be less than 100 which is a conservative estimate of its value *in vivo*.

Whatever the synthetic mechanism involved, her conclusion that malate is accumulated in a site remote from that of its synthesis appears to be inescapable. The constancy of the ^{14}C distribution in malate that accumulates when $^{14}CO_2$ is supplied (see p. 101), compared with the readiness with which ^{14}C present in malate fed to the leaves becomes randomized, argues for the occurrence of accumulation at some metabolically inert site. Moreover, the magnitude in itself suggests that the malate does not accumulate in the cytoplasm. From data obtained in Vickery's laboratory on maximum levels of malate in *Bryophyllum* leaves, and on the basis of certain assumptions, Stiller has calculated the concentrations of acid which would occur in the cells. If the cytoplasm occupied one per cent and the vacuole 30 per cent of the total cell volume, the concentration in the cytoplasm would be about 7 *M;* in the vacuole the concentration would be at the more acceptable value of 0.25 *M*.

The effects of CO_2 concentration on malate synthesis *in vitro* by the PEP carboxylase-malic dehydrogenase system (107) and by malic enzyme (105) provide evidence that it is the former system which is involved in leaves which show CAM. Walker & Brown (107) stressed the property observed previously (5, 84) that PEP carboxylase has a high affinity for CO_2 and that this would account for the occurrence of acidification in Crassulacean leaves even in the absence of atmospheric CO_2. The rate of malate synthesis by Reactions 9 and 10 was found to increase with CO_2 concentration up to 0.5 per cent. Between 0.5 and 10 per cent the rate fell slowly to about two-thirds of the maximum, and increases above 10 per cent resulted in progressive inhibitions. The component enzyme affected was the PEP carboxylase. In contrast, with malic enzyme, Walker (105) found the carboxylation of pyruvate to be slow at low concentrations of CO_2 and to increase progressively to a maximum at about 30 per cent CO_2. Both of the enzymic systems studied were extracted from *Kalanchoë* leaves and it is clear that whereas the effects of CO_2 concentration on the PEP carboxylase system show some similarity to the effects of CO_2 concentration on acidification of the intact leaves (92), the effects on malic enzyme bear no such relation; maximum acid synthesis was measured in 30 per cent CO_2 in which acidification of the intact leaves is retarded.

A problem yet to be solved is the source of the reduced pyridine nucleotide consumed when malate is accumulated *in vivo*. The participation of PEP carboxylase and malic dehydrogenase would require $DPNH_2$; participation of malic enzyme would require $TPNH_2$. The proposed pentose carboxylation pathway for malate synthesis (see p. 101) would possibly generate $TPNH_2$ in the production of RDP from hexose, whereas in the subsequent reduction of oxaloacetate to malate $DPNH_2$ would be required. The substitution of malic enzyme for PEP carboxylase and malic dehydrogenase in the sequence would simplify the question of coenzyme linkage but would raise other problems. The production of PEP by way of the glycolytic pathway would generate $DPNH_2$, but a synthetic pathway for malate in which PEP produced in this way is converted into malate by PEP carboxylase and malic dehydro-

genase does not involve the double CO_2 fixation apparently required (p. 101). Many difficulties are overcome, however, if a mechanism for the transfer of hydrogen between DPN and TPN is present in the leaves, or if the reduced pyridine nucleotide, involved in the reductive carboxylation which yields malate, is generated in reactions other than those which produce the CO_2 acceptor from carbohydrate.

Malic enzyme may well be mainly concerned in malic consumption in CAM, as also may be PEP carboxykinase if it is present. The former would yield pyruvate. The latter would yield PEP and, as suggested by Vennesland *et al.* (47, 84), thus facilitate the conversion of acids into carbohydrate, particularly in the light. Synthesis of carbohydrate from pyruvate by direct reversal of glycolysis including the phosphopyruvic kinase reaction cannot yet, however, be discounted (e.g., 34).

Apart from other considerations, the functioning of the isocitric enzyme system (Eq. 1) *in vivo* as a mechanism for acid synthesis with CO_2 fixation would require a source of α-oxoglutarate independent of that from the TCA cycle. Breakdown of nitrogenous components by way of glutamate would supply the keto acid but there is no evidence of the occurrence of such reactions in the normal course of acid synthesis in CAM (45).

The tricarboxylic acid cycle:—No active preparations of mitochondria reputed to house the component enzymes of the cycle in other tissues, including leaves (76), have yet been made from Crassulacean leaves. Nevertheless it appears probable that the cycle is operative in them (16, 75). Bradbeer (16), for example, observed that in *Kalanchoë* leaves most of the TCA intermediates are present and that changes in the level of malate are followed in turn by changes in the level of citrate and α-oxoglutarate. Moreover the incorporation of ^{14}C into malate is rapidly followed by its spread into tricarboxylic acids and α-oxoglutarate. Succinate is not appreciably labelled but this is to be expected as the label in malate is confined to the carboxylic groups (p. 101). In Bradbeer's experiments the specific activities of malate, citrate, and α-oxoglutarate measured after 9 hr. in the dark were very similar, suggesting complete equilibration between them. The specific activity of isocitrate was markedly lower than that of the other acids. This was attributed to the presence of much of the isocitrate in a storage pool which equilibrated rather slowly with the pool of acid in contact with cycle enzymes. Presumably much of the malate and citrate are in storage pools also. For citrate, equilibration between pools must be more rapid than in the case of isocitrate. Saltman *et al.* (75) have pointed out that the probable existence of acids in active and storage pools would explain some of the earlier doubts about the operation of the TCA cycle in tissues showing CAM. These included the apparently sluggish equilibration of label between malate and cycle acids (70, 83) and the great divergence between the isocitrate/citrate ratios in leaves (54, 70) and the ratio observed when these acids are in equilibration with aconitase *in vitro* (38, 46).

Supporting evidence for the operation of the cycle in *Kalanchoë* is plenti-

ful. Specifically labelled pyruvic and fumaric acids infiltrated into the leaves are rapidly consumed with a distribution of label through cycle acids in accord with the classic cycle mechanism (17, 18). Arsenite, malonate, and fluoroacetate at low concentrations result in reduced oxygen uptake and accumulations of cycle intermediates (see p. 91 & 93). High CO_2 concentrations known to inhibit succinic oxidase *in vitro* (10, 73) result in succinate accumulation in the leaves (71).

Moyse *et al.* (25, 54, 55) adhere to the view that though the cycle operates to a degree in leaves of *B. daigremontianum,* partial reactions involved in the consumption of isocitrate appear to be retarded relative to those involved in its production. They suggested (54) that isocitrate may be formed independently of cycle reactions, for example, by carboxylation of α-oxoglutarate, and (25) that the accumulation of fumarate generated from malate by fumarase might account for the slow rate of the cycle as a whole. Ramstad & Lieberman (70) postulated the existence of a dicarboxylic acid cycle in *Bryophyllum* and the synthesis of tricarboxylic acids by combination of a dicarboxylic acid with glyoxylic acid. Subsequent feeding experiments with glycollate (44) provided no support for the suggestion.

Other enzymic systems:—There is no direct evidence of the occurrence of the glyoxylate cycle (37) in leaves showing CAM. However, when Wood (115) observed that, in *Sedum* leaves under certain conditions, isocitrate frequently fluctuated in the opposite sense to malate, without the CO_2 output to be expected if isocitrate were consumed by TCA-cycle reactions, she suggested the possibility that another pathway is involved. Participation of the glyoxylate cycle would be one explanation.

The appearance of labelled aspartate, glutamate, and alanine among products of dark $^{14}CO_2$ fixation has been attributed to transaminations involving the corresponding labelled keto acids (24, 75, 92). The presence of transaminases has been demonstrated in extracts from *Bryophyllum* (26) and *Kalanchoë* (108). Enzymic mechanisms by which ^{14}C may be incorporated into other amino acids in leaves of these plants have been discussed (24, 43, 75).

Ehrenberg (28) has observed a daily rhythm in phosphatase activity in leaves of *B. blossfeldiana.* Its relevance to CAM is at present unknown.

Malic Acid Synthesis *in Vivo*

The incorporation of $^{14}CO_2$ in the dark:—The conclusion that malic acid is the first product which accumulates as a result of dark CO_2-fixation in tissues showing CAM, was first substantiated in experiments by Stutz & Burris (83), Thurlow & Bonner (93), and Varner & Burrel (94), using $^{14}CO_2$, and has been amply verified since by similar experiments in a number of laboratories (16, 55, 74, 92). The observations did not, however, rule out the possibility that there are products of CO_2-fixation that precede malate in the reaction sequence and are either present in trace amounts or too un-

stable to survive the analytical procedures employed. One compound of particular interest in this category is oxaloacetate because its production before malate would provide evidence that the PEP carboxylase-malic dehydrogenase system rather than malic enzme is operative in the CO_2-fixation.

Saltman *et al.* (74) detected only two labelled compounds, malate and aspartate, as products of dark $^{14}CO_2$ fixation after 5 sec., in young *Bryophyllum* leaves. The amounts of ^{14}C in each of these compounds, as percentages of the total ^{14}C incorporation, were less after 1 min. and still less after 5 min. and they concluded therefore that malate and aspartate might well have originated from a common precursor. Since they were able to isolate labelled oxaloacetate from the products of longer periods of dark fixation (74, 75) and to detect PEP carboxylase in extracts from the leaves they concluded that the "first product" of CO_2-fixation was oxaloacetate. Evidence discussed earlier (p. 98) suggests that CO_2-fixation in mature *Kalanchoë* leaves is mediated by PEP carboxylase and hence that oxaloacetate would be formed before malate. Even so it may not be the first product of CO_2-fixation in these leaves. In dark CO_2-fixation by the postulated pentose carboxylation pathway (p. 101), the production of PGA (phosphoglyceric acid) would precede the production of oxaloacetate. Phosphoglyceric acid has been identified among the products of dark CO_2-fixation (see p. 102).

Bradbeer, Ranson & Stiller (19) have summarized data from various sources which suggest that in malic acid synthesized from $^{14}CO_2$ in the dark in tissues showing CAM, the label is confined to the carboxylic groups, with two-thirds in the C-4 and one-third in the C-1 position. The distribution observed in *Kalanchoë* and *Bryophyllum* leaves over a number of years was the same for fixation periods from 4 sec. to 24 hr. duration at several temperatures. Moreover, it remained unchanged when, after the period of $^{14}CO_2$ fixation, leaves were retained in air in the dark for prolonged periods, or transferred to the light. Jolchine (36) has confirmed some of these observations for *B. daigremontianum*. Bradbeer *et al.* contrasted the apparently constant ^{14}C distribution in the malate in Crassulacean leaves with that occurring in malate labelled by dark $^{14}CO_2$ fixation in some tissues which do not show CAM. In the latter the label was distributed in varying proportions between the carboxylic groups.

The pentose carboxylation pathway of malate synthesis:—Bradbeer *et al.* (19) pointed out that if malate produced in CAM is synthesized by β-carboxylation, ^{14}C would first appear in carbon 4 of the malate. Metabolism of malate by way of the TCA cycle would produce only unlabelled malate and so would not modify the initial pattern of labelling. They dismissed also the possibility that randomization by either fumarase or enzymes of the glyoxylate bypass (37) could account for the constancy of the labelling pattern and suggested that, in malate synthesis in tissues showing CAM, Reaction 13 catalysed by RDP carboxylase, and Reaction 14 catalysed by enolase,

may precede Reactions 9 and 10. There would thus be two carboxylations (Reactions 13 and 9) in malate synthesis and the observed distribution of ^{14}C

$$RDP + CO_2 \longrightarrow 2\ PGA \qquad 13.$$

$$2\ PGA \longrightarrow 2\ PEP \qquad 14.$$

in malate would be imposed at the time of synthesis and not by reactions subsequent to synthesis.

Operation of the pathway in CAM would require the presence in the tissues of a mechanism for producing RDP in the dark. Pentose phosphate could be derived from hexose in reactions involving oxygen uptake and CO_2 output with an RQ of unity by way of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, or without gas exchange in reactions involving transketolase and transaldolase. Either way, the computations of CO_2 fixed in acid synthesis made in earlier physiological experiments (see p. 93) remain valid within the limits stated. Provided then that the reduced pyridine nucleotide consumed in malate synthesis is produced in the normal course of respiratory oxidations, it can be shown that the data on gas uptake fit as well the operation of the pentose carboxylase pathway as the single carboxylation pathway (see p. 93) in malate synthesis.

The first evidence that PGA may be produced in dark CO_2-fixation came when Stiller (80) detected a labelled substance with many of its properties in *Bryophyllum* leaves after exposure to $^{14}CO_2$ for several hours. Subsequent attempts by Stiller (81) to isolate labelled PGA after very short fixation periods were not uniformly successful, but convincing evidence was produced of its occurrence after a 15 sec. period of dark fixation. Kunitake *et al.* (43) have detected labelled PGA in young *Bryophyllum* leaves exposed for 5 min. to $^{14}CO_2$ in the dark, but not among the products of shorter exposures. As they state, however, it is possible that the age or other physiological condition of the leaves may account for the difference in their observations (cf. p. 87).

To test further the pentose carboxylation hypothesis, Stiller incubated discs of *Bryophyllum* leaf for 3 hr. with gluconate-^{14}C, glucose-1-^{14}C, glucose-2-^{14}C and ribose-1-^{14}C in the dark and degraded the malic acid produced. That produced from the gluconate contained label in the central carbon atoms as well as in the carboxylic groups. The distributions of ^{14}C in malate produced from the labelled sugars were complex. All three yielded large percentages of label in the carboxylic groups but the low degree of asymmetry between that in C–1 and C–4 suggested to Stiller that extensive sugar transformations occurred in the leaves. Taking these into account, the results as a whole were considered to be consistent with participation of the pentose carboxylation pathway in malate synthesis.

Special features of malic acid synthesis in CAM:—A wide variety of plant tissues are known to incorporate $^{14}CO_2$ in the dark and in, at least, the majority the first stable product to accumulate label is malic acid. This is in

accord with the wide distribution of malic enzyme and PEP carboxylase (p. 97). Substances labelled after malate are largely confined to acids of the TCA cycle and amino acids derived from them and, apart from those tissues (15, 82) in which fat is being incorporated into carbohydrate and in which the glyoxylate cycle (37) is operative, there is little, if any, incorporation of ^{14}C into carbohydrates. Thus as far as the pattern of labelled products is concerned, perhaps the only difference in tissues showing CAM is that redistribution of the label from malate is slower than in other tissues. What may, possibly, prove to be a major difference is the characteristic and constant pattern of the label observed in the malate synthesized in Crassulacean leaves. It is clear that such a constant label distribution does not exist in the malate labelled when all plants are exposed to $^{14}CO_2$ in the dark, but it is not yet proved that the labelling pattern observed in the Crassulacean leaves never occurs in tissues which do not show CAM. The enzymic systems involved in the pentose carboxylation pathway, postulated to account for the label distribution in tissues showing CAM, are possibly present in all green tissues. On these grounds there is no reason why malate synthesis by such a mechanism should be confined to tissues showing CAM unless there is some special association of the enzymes, spatial or otherwise, which facilitates the process in the latter.

Apart from the synthetic pathway, however, the constancy of the labelling pattern also carries the requirement for storage at a site remote from sites of production and consumption, and the requisite transport mechanisms for malic acid. A further possibility is that there exists in tissues showing CAM, but not to the same extent in all plants, metabolic systems which couple closely with the acid synthesis system and maintain the supply of reduced pyridine nucleotide which is essential for malate synthesis.

Malate synthesis in the light:—CO_2-feeding experiments with leaves in the light (p. 85) suggest that whatever the process involved in malic acid synthesis in the dark it can operate in basically the same way in the light, but that under natural conditions the CO_2 available is preferentially consumed in photosynthesis. Tracer studies by Norris & Calvin (57, 58) and others suggest that the pathway by which CO_2 is assimilated into carbohydrate in bright light and at normal temperatures and CO_2 concentrations is not basically different in *Kalanchoë* and *Bryophyllum* leaves from that in other green tissues. The proportion of $^{14}CO_2$ incorporated into acids, in the light in tissues showing active CAM may, however, be greater than that which occurs in other green tissues. Thus, in one experiment in which leaves of *B. daigremontianum* were illuminated at saturating light intensity (20,000 lux) in air containing $^{14}CO_2$ (the total CO_2 concentration was 0.04 per cent) for 6 min., Moyse & Jolchine (55) found that about 20 per cent of the ^{14}C fixed was present in acids. Malic acid contained most of the ^{14}C and small amounts were present in citric, fumaric, glyceric and glycollic acids. The presence of label in the latter two acids was noted as the major difference between acid products of light and dark $^{14}CO_2$ fixation (cf. p. 102).

In other experiments in an atmosphere containing 0.12 per cent CO_2, Moyse *et al.* (25, 55) compared $^{14}CO_2$ incorporation in the dark and in low and high light intensities. With increase in light intensity, the total ^{14}C incorporation increased (about twentyfold at 20,000 lux) as did also the proportion of label in insoluble products, sugars, phosphorylated sugars and amino acids. The proportion of ^{14}C in the acid fraction decreased from 90 per cent in the dark to 20 per cent in bright light, but the total activity and specific activity of individual acids increased with light intensity. Moyse *et al.* (25, 55) concluded that β-carboxylation continued in the light simultaneously with the carboxylation of RDP, and attributed the increased specific activity of malic acid in the light to its production from PEP labelled already as a result of photosynthetic CO_2 fixation. The presence of label in the central carbon atoms as well as in the carboxylic groups of malate synthesized in the light (36) supports this view, though there well may be other factors involved. The enhanced activities of citric, isocitric, and glutamic acids in the light were attributed to an increased rate of cytoplasmic oxidation (see p. 106).

Carbon dioxide feeding experiments (21, 72, 89) with *Kalanchoë* leaves in the light have shown that though acidification is most marked in about 5 per cent CO_2 it continues with relatively little retardation in CO_2 concentrations in the range 10 to 20 per cent in which photosynthetic CO_2 uptake is markedly inhibited, i.e., CO_2 uptake (associated with acid synthesis) continued but the oxygen output was near zero. Conclusions from gaseous exchange have been confirmed by use of $^{14}CO_2$. One interpretation of these observations is that CO_2-fixation into acids proceeds by mechanisms independent in the main of those involved in photosynthetic CO_2-fixation. An alternative interpretation, for which there is a little supporting evidence, is that it is not the carboxylation of RDP which is inhibited by the CO_2 but the reduction of PGA to carbohydrate, possibly by an effect of CO_2 on a reaction producing the necessary "assimilatory power".

The suggestion has been made by Kunitake & Saltman (41) that light acidification occurs in leaves showing CAM when, by CO_2 feeding, the RDP carboxylase pathway for CO_2 fixation is saturated. An alternative suggestion is that it occurs when the assimilatory system which converts PGA to carbohydrate is saturated. Detailed discussion of the possible causes of enhanced acidification in the light and also in the dark must be deferred until more is known about the effects of CO_2 concentration on the RDP carboxylase system.

The Consumption of Malic Acid *in Vivo*

Since, at present, it appears to be improbable that the consumption of malate involves the incorporation of the carbon skeleton *en bloc* into some other compound, the first step probably involves a decarboxylation to yield CO_2 and either pyruvate or PEP. Two fates are then possible for the 3-carbon residue, namely oxidation by way of the TCA cycle, or incorporation into carbohydrate by way of the glycolytic pathway. One or both

of these processes coupled with the photosynthetic assimilation of CO_2 would account for the incorporation of malate carbon into carbohydrate in the light (p. 105). Carbohydrate synthesis during dark deacidification (p. 84) would presumably utilize only the pyruvate or PEP.

Consumption in the dark:—In tissues showing CAM, after all but the shortest periods of $^{14}CO_2$ fixation in the dark, acids of the TCA cycle and derived amino acids are present among the labelled products (16, 24, 75, 83, 92, 94, 115). Almost without exception the various investigators have attributed at least some of the label in these acids to consumption of malate or oxaloacetate in TCA cycle reactions. It may therefore be concluded that some of the malic acid is oxidatively consumed. Evidence from these experiments of consumption in other ways is lacking. One-third of the label in the malate is present in the α-carboxylic group (p. 101) and would be incorporated into any carbohydrate synthesized from PEP or pyruvate. There is, however, no evidence yet that $^{14}CO_2$ supplied to leaves in the dark is eventually incorporated, except possibly in trace amounts, into carbohydrate. The range of environmental and physiological conditions so far investigated is, unfortunately, very narrow.

Bradbeer *et al.* (17, 18) found that specifically labelled pyruvic and fumaric acids infiltrated into *Kalanchoë* leaves in the dark at 16°C. were for the most part consumed oxidatively by way of the TCA cycle. Nevertheless after 8 hr. about 5 per cent of the label in the pyruvate and one per cent of that in the fumarate were recovered in free sugars. Since pyruvate-1-^{14}C was almost as effective as pyruvate labelled in the 2 and 3 positions in producing labelled sugars, the simplest explanation is that the label reached the sugars by the reversal of glycolysis. The distribution of ^{14}C in malic acid produced from pyruvate indicated some incorporation of the pyruvate into dicarboxylic acids by β-carboxylation, hence the possibility exists that the infiltrated acids were converted by way of oxaloacetate into PEP as a preliminary step. Synthesis of carbohydrate from acid cannot be inferred, but a mechanism for transferring label from acids to carbohydrate obviously exists in *Kalanchoë* leaves. One possibility is that when dark deacidification is slow, acids are consumed oxidatively as fast as they are released into the cytoplasm. A corollary stating that when dark deacidification is extremely rapid, as for example at high temperatures, label from malic acid finds its way into carbohydrate, has not yet been fully investigated.

Consumption in the light:—When leaves of *B. calycinum* were illuminated after a period in which $^{14}CO_2$ was fixed in the dark, Varner & Burrell (94) observed that labelled carbon gradually found its way from acids into carbohydrate. In the course of this transfer the activities of citrate and isocitrate increased relative to the activity in malate, suggesting cyclic consumption of malate. In hexose derived from the starch which became labelled, ^{14}C appeared first in carbon atoms 3 and 4, which rules out only the possibility that the 4-carbon skeleton of malate is incorporated directly into carbohydrate in the light. Moyse *et al.* (25, 53) in basically similar experiments

with leaves of *B. daigremontianum,* observed that the utilization of endogenous malic acid in photosynthesis is much slower than utilization of $^{14}CO_2$ from the atmosphere. From measurements of total and specific activities of malic, citric, isocitric, and glutamic acid, they concluded that malate is consumed oxidatively, and they suggested that the incorporation of malic acid into carbohydrate may involve, as the first step, its complete oxidation to CO_2. Moreover, as a result of their comparative studies in the dark and in low and high light intensities (see p. 103) Moyse *et al.* (25, 55) have suggested that the cytoplasmic oxidation of malate is faster in the light than in the dark. Recognizing that their suggestion is, apparently, at variance with an earlier conclusion (11, 23, but see also 48) that oxidation of metabolites by way of the TCA cycle is inhibited in the light, they point out that the leaves they used show strong β-carboxylase activity. Noting also that the inferred inhibition by light of oxidative reactions may occur only near chloroplasts, and that there is evidence [Brown (20)] that the overall oxygen uptake of tissues is unaffected by light, they conclude that cytoplasmic oxidations in parts of the cell remote from chloroplasts may in fact be enhanced in the light. They suggest that the oxygen liberated in green cells in the light may be responsible for the enhanced oxidative activity.

In the observation (see p. 101) that the distribution of label imposed when malic acid is synthesized in the dark from $^{14}CO_2$ remains unaltered in leaves subsequently exposed to light, Moyse *et al.* (53) see evidence for the conclusion that in the light as in the dark the equilibrium *in vivo* of the reaction, PGA $\rightleftharpoons$ PEP, is towards the formation of PEP (cf, however, p. 97). They suggest that this makes unlikely the direct reutilization of PEP in the synthesis of sugars. Haidri (32) has shown, however, that when 2-^{14}C pyruvate and 2-^{14}C malate are infiltrated into *Bryophyllum* leaves in the light, carbohydrates incorporate ^{14}C. In the hexose prepared from starch isolated after 3 hr., 28 to 33 per cent of the ^{14}C was present in carbon atoms 3 plus 4, 43 to 46 per cent in C_2 plus C_5 and 25 to 28 per cent in C_1 plus C_6. That most of the ^{14}C was present in C_2 plus C_5 suggests that some of the pyruvate was incorporated into sugar by reversal of glycolysis. The presence of ^{14}C also in C_3 plus C_4 of the hexose indicates that some of the acid was oxidized to yield $^{14}CO_2$ which was then assimilated. These observations provide support for the view that light deacidification involves the complete oxidation of malic acid, but they suggest also that it may be consumed in other ways.

Kunitake *et al.* (41) observed that in the course of the conversion of acids, labeled by dark fixation of $^{14}CO_2$, into phosphorylated and free sugars in the light, the total amount of ^{14}C present in *Bryophyllum* leaves remained unchanged. Since, moreover, they were unable to detect any $^{14}CO_2$ liberation into the atmosphere around the leaves, they concluded that CO_2 liberated on decarboxylation of the acids entered directly into the light fixation mechanism. It is of note, however, that on occasions the CO_2 released from acids in the light may be greater than that which can be consumed immediately

in photosynthesis. There are reports (e.g., 51) of CO_2 liberation for a period when leaves which have accumulated high concentrations of acid are first transferred into bright light. Gregory *et al.* (see p. 87) have observed also that CO_2 is liberated at times from *Bryophyllum* leaves in the light.

LITERATURE CITED

1. Anderson, D. G., Stafford, H. A., Conn, E. E., and Vennesland, B., *Plant Physiol.*, **27**, 675–84 (1952)
2. Avadhani, P. N., *Studies on the Fixation of Carbon Dioxide in Leaves and Roots of Kalanchoë and Roots of Ricinus* [Doctoral thesis, Durham Univ., Durham, England (Newcastle Division) 1957]
3. Axelrod, B., and Bandurski, R. S., *J. Biol. Chem.*, **204**, 939–48 (1953)
4. Axelrod, B., and Beevers, H., *Ann. Rev. Plant Physiol.*, **7**, 267–98 (1956)
5. Bandurski, R. S., *J. Biol. Chem.*, **217**, 137–50 (1955)
6. Bandurski, R. S., and Greiner, C. M., *J. Biol. Chem.*, **204**, 781–86 (1953)
7. Beevers, H., and Gibbs, M., *Plant Physiol.*, **29**, 322–24 (1954)
8. Bennet-Clark, T. A., *Ann. Rev. Biochem.*, **18**, 639–54 (1949)
9. Bennet-Clark, T. A., *New Phytologist*, **32**, 37–71, 128–61, 197–230 (1933)
10. Bendall, D. S., Ranson, S. L., and Walker, D. A., *Nature*, **181**, 133–34 (1958)
11. Benson, A. A., and Calvin, M., *J. Exptl. Botany*, **1**, 63–8 (1950)
12. Bharucha, F. R., and Joshi, G. V., *J. Biol. Sci. (Bombay)* **1**, 5–12 (1958)
13. Bharucha, F. R., and Joshi, G. V., *J. Univ. Bombay*, **25**, 23–8 (1957)
14. Bonner, W. D., and Bonner, J., *Am. J. Botany*, **35**, 113–17 (1948)
15. Bradbeer, C., *Nature*, **182**, 1429–30 (1958)
16. Bradbeer, J. W., *Some Carbohydrate and Acid Conversions in Kalanchoë Crenata* [Doctoral thesis, Durham Univ., Durham, England (Newcastle Division) 1954]
17. Bradbeer, J. W., *Malate Synthesis in Crassulacean Leaves* (112th Conf., Soc. Exptl. Biol., Univ. College of Swansea, Swansea, Wales, 1959)
18. Bradbeer, J. W., and Ranson, S. L., *The Conversion of Acids into Carbohydrates in Kalanchoë Leaves in the Dark* (109th Conf., Soc. Exptl. Biol., King's College, Newcastle upon Tyne, England, 1958)
19. Bradbeer, J. W., Ranson, S. L., and Stiller, R. M., *Plant Physiol.*, **33**, 66–70 (1958)
20. Brown, A. H., *Am. J. Botany*, **40**, 719–29 (1953)
21. Brown, J. M. A., *Studies of Inhibitors in Relation to Crassulacean Acid Metabolism* [Doctoral Thesis, Durham Univ., Durham, England (Newcastle Division) 1956]
22. Bruinsma, J., *Studies on Crassulacean Acid Metabolism*, 531–90 (Academisch Proefschrift. North Holland Publishing Co., Amsterdam, The Netherlands, 1958)
23. Calvin, M., and Massini, P., *Experientia*, **8**, 445–57 (1952)
24. Champigny, M. L., *Compt. rend.*, **243**, 83–5 (1956)
25. Champigny, M. L., Jolchine, C., and Moyse, A., *Radioisotopes in Scientific Research*, **4**, 458–67; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, 690 pp., 1958)
26. Coles, C. H., and Waygood, E. R., *Can. J. Botany*, **35**, 25–30 (1957)
27. Doorenbos, J., and Wellensiek, S. J., *Ann. Rev. Plant Physiol.*, **10**, 147–84 (1959)

28. Ehrenberg, M., *Planta,* **43,** 528–36 (1954)
29. Evans, H., *Biol. Revs. Biol. Proc. Cambridge Phil. Soc.,* **7,** 181–211 (1932)
30. Gibbs, M., and Beevers, H., *Plant Physiol.,* **30,** 343–7 (1955)
31. Gregory, F. G., Spear, I., and Thimann, K. V., *Plant Physiol.,* **29,** 220–9 (1954)
32. Haidri, S. Z. A., *Plant Physiol.,* **30,** Suppl. 4, iv (1955)
33. Harary, I., Korey, S. R., and Ochoa, S., *J. Biol. Chem.,* **203,** 595–604 (1953)
34. Hiatt, H. H., Golstein, M., Lareau, J., and Horecker, B. L., *J. Biol. Chem.,* **231,** 303–7 (1958)
35. Jackson, W. A., and Coleman, M. T., *Plant and Soil,* **11,** 1–16 (1959)
36. Jolchine, G., *Bull. soc. chim. biol.,* **41,** 227–34 (1959)
37. Kornberg, H. L., and Krebs, H. A., *Nature,* **179,** 988–91 (1957)
38. Krebs, H. A., and Eggleston, L. V., *Biochem. J.,* **37,** 334–38 (1943)
39. Krebs, H. A., and Eggleston, L. V., *Biochem. J.,* **38,** 426–37 (1944)
40. Krotkov, G., Runeckles, V. C., and Thimann, K. V., *Plant Physiol.,* **33,** 289–92 (1958)
41. Kunitake, G. M., and Saltman, P., *Plant Physiol.,* **33,** 400–3 (1958)
42. Kunitake, G. M., Saltman, P., and Lang, A., *Plant Physiol.,* **32,** 201–3 (1957)
43. Kunitake, G. M., Stitt, C., and Saltman, P., *Plant Physiol.,* **34,** 123–27 (1959)
44. Lieberman, H., Ramstad, E., and Christian, J. E., *J. Am. Pharm. Assoc. Sci. Ed.,* **45,** 295–300 (1956)
45. Lyndon, R. F., *Studies on the Nitrogen Metabolism of Kalanchoë Leaves in the Dark in Relation to Acidification and Deacidification and Gaseous Exchange* [Doctoral thesis, Durham Univ., Durham, England (Newcastle Division) 1957]
46. Martius, C., and Leonhardt, L., *Hoppe-Seyler's Z. physiol. Chem.,* **278,** 208–12 (1943)
47. Mazelis, M., and Vennesland, B., *Plant Physiol.,* **32,** 591–600 (1957)
48. Milhaud, G., Benson, A. A., and Calvin, M., *J. Biol. Chem.,* **218,** 599–606 (1956)
49. Moyle, J., and Dixon, M., *Biochim. et Biophys. Acta.,* **16,** 434–35 (1955)
50. Moyse, A., *Physiol. Plantarum,* **8,** 453–77 (1955)
51. Moyse, A., *Physiol. Plantarum,* **8,** 478–92 (1955)
52. Moyse, A., *Compt. rend.,* **233,** 1656-8 (1951)
53. Moyse, A., Champigny, M. L., Dardart, J., and Jolchine, G., *Intern. Abstr. Biol. Sci.,* Sect. II, p. 138, Intern. Congr. Biochem., 4th Congr., Vienna, Austria (1958)
54. Moyse, A., and Jolchine, G., *Bull. soc. chim. biol.,* **38,** 761–84 (1956)
55. Moyse, A., and Jolchine, G., *Bull. soc. chim. biol.,* **39,** 725–45 (1957)
56. Neyland, M., and Thimann, K. V., *Plant Physiol.,* **31,** Suppl., 35–6 (1956)
57. Norris, L., and Calvin, M., *Ann. Acad. Sci., Fennicae,* **60,** 32–36 (1955)
58. Norris, L., Norris, R. E., and Calvin, M., *J. Exptl. Botany,* **6,** 64–74 (1955)
59. Nossal, P. M., *Biochem. J.,* **50,** 349–55 (1952)
60. Ochoa, S., *J. Biol. Chem.,* **159,** 243–44 (1945)
61. Ochoa, S., Mehler, A., and Kornberg, A., *J. Biol. Chem.,* **167,** 871–72 (1947)
62. Overbeck, G., *Protoplasma,* **48,** 241–60 (1957)
63. Paxton, R. G., *Studies on the Influence of Carbon Dioxide on the Acid and Zymasic Metabolism of Plants* [M.Sc. thesis, Durham Univ., Durham, England (Newcastle Division) 1952]

64. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **22,** 1–19 (1947)
65. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **22,** 205–27 (1947)
66. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **22,** 360–76 (1947)
67. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **22,** 477–93 (1947)
68. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B., *Plant Physiol.*, **23,** 123-32 (1948)
69. Pucher, G. W., Vickery, H. B., Abrahams, M. D., and Leavenworth, C. S., *Plant Physiol.*, **24,** 610–20 (1949)
70. Ramstad, E., and Lieberman, H., *Medd. Norsk Farm, Selskap,* **17,** 214–25 (1955)
71. Ranson, S. L., *Nature,* **172,** 252 (1953)
72. Ranson, S. L., *Rappts. Communs.,* Sect. II, 60–3 (Congr. intern botan. 8e Congr., Paris, France, 1954)
73. Ranson, S. L., Walker, D. A., and Clarke, I. D., *Biochem. J.,* **66,** 57P (1957)
74. Saltman, P., Kunitake, G., Spolter, H., and Stitts, C., *Plant Physiol.,* **31,** 464–68 (1956)
75. Saltman, P., Lynch, V. H., Kunitake, G. M., Stitts, C., and Spolter, H., *Plant Physiol.,* **32,** 197–200 (1957)
76. Smillie, R. M., *Australian J. Biol. Sci.,* **9,** 81–91 (1956)
77. Somers, G. F., *Plant Physiol.,* **26,** 1–18 (1951)
78. Spear, I., and Thimann, K. V., *Plant Physiol.,* **29,** 414–17 (1954)
79. Spoehr, H. A., *The Carbohydrate Economy of the Cacti, Publ. 287* (Carnegie Inst. Washington, Washington, D.C., 1919)
80. Stiller, M. L., *Experiments on the Relationship Between Succulence and Acid Accumulation in Bryophyllum, with an Addendum on the Mechanism of Acid Accumulation* (M.Sc. thesis, Purdue Univ., Lafayette, Ind., 1956)
81. Stiller, M. L., *The Mechanism of Malate Synthesis in Crassulacean Leaves* (Doctoral thesis, Purdue Univ., Lafayette, Ind., 1959)
82. Stiller, M. L., Neal, G. E., and Beevers, H., *Plant Physiol.,* **33,** Suppl. 34 (1958)
83. Stutz, R. E., and Burris, R. H., *Plant Physiol.,* **26,** 226–43 (1951)
84. Tchen, T. T., and Vennesland, B., *J. Biol. Chem.,* **213,** 533–46 (1955)
85. Thoday, D., and Jones, K. M., *Ann. Botany (London),* **3,** 677–98 (1939)
86. Thoday, D., and Richards, K. M., *Ann. Botany (London),* **8,** 189–203 (1944)
87. Thomas, M., *Plant Physiology,* 3rd ed. (J. & A. Churchill, Ltd., London, England, 504 pp., 1947)
88. Thomas, M., *New Phytologist,* **48,** 390–420 (1949)
89. Thomas, M., *Symposia Soc. Exptl. Biol.,* **5,** 72–93 (1951)
90. Thomas, M., and Beevers, H., *New Phytologist,* **48,** 421–47 (1949)
91. Thomas, M., and Fidler, J. C., *New Phytologist,* **40,** 217–39 (1941)
92. Thomas, M., and Ranson, S. L., *New Phytologist,* **53,** 1–30 (1954)
93. Thurlow, J., and Bonner, J., *Arch. Biochem.,* **19,** 509–11 (1948)
94. Varner, J. E., and Burrell, R. C., *Arch. Biochem.,* **25,** 280–7 (1950)
95. Vennesland, B., Ceithaml, J., and Gollub, M., *J. Biol. Chem.,* **171,** 445–6 (1947)
96. Vennesland, B., and Conn, E. E., *Ann. Rev. Plant Physiol.,* **3,** 307–32 (1952)

97. Vickery, H. B., *Plant Physiol.*, **27**, 9–17 (1952)
98. Vickery, H. B., *Plant Physiol.*, **27**, 231–39 (1952)
99. Vickery, H. B., *Plant Physiol.*, **29**, 385–92 (1954)
100. Vickery, H. B., *Plant Physiol.*, **29**, 520–26 (1954)
101. Vickery, H. B., *Plant Physiol.*, **31**, 455–64 (1956)
102. Vickery, H. B., *Plant Physiol.*, **32**, 220–24 (1957)
103. Walker, D. A., *Enzymically Active Extracts from Crassulacean Plants and Their Relationship to Dark Acidification* [Doctoral thesis, Durham University, Durham, England (Newcastle Division) 1956]
104. Walker, D. A., *Biochem. J.*, **67**, 73–79 (1957)
105. Walker, D. A., *Biochem. J.* (In press)
106. Walker, D. A., *Nature*, **178**, 593–4 (1956)
107. Walker, D. A., and Brown, J. M. A., *Biochem. J.*, **67**, 79–83 (1957)
108. Walker, D. A., and Ranson, S. L., *Plant Physiol.*, **33**, 226–30 (1958)
109. Whatley, F. R., *New Phytologist*, **50**, 258–67 (1951)
110. Wolf, J., *Planta*, **15**, 572–644 (1931)
111. Wolf, J., *Planta*, **28**, 60–86 (1938)
112. Wolf, J., *Planta*, **29**, 450–67 (1939)
113. Wolf, J., *Planta*, **37**, 510–34 (1949)
114. Wolf, J., in *Encyclopedia of Plant Physiology*, **12** (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, In press)
115. Wood, W. M. L., *J. Exptl. Botany*, **3**, 336–55 (1952)
116. Zabka, G., Gregory, F. G., and Edelman, J., *Nature*, **183**, 1375–77 (1959)

HEAT TRANSFER BETWEEN THE PLANT AND THE ENVIRONMENT[1]

By K. Raschke
Berlin, Germany

Analysis of Heat Transfer and Environment

The Law of the Conservation of Energy, which at the time of its discovery was very much derided, has since become so generally accepted that the terms "heat" and "energy" can be substituted for one another without the necessity of further explanation. Energy manifests itself in several physical forms, therefore it is reasonable to expect many forms of heat transfer between a plant and its environment.

Thermodynamics teaches that in order to have a heat flow there must be an energy gradient. This gradient may take the more specific aspect of a temperature, pressure, or concentration gradient. In any area being considered, if the amount of energy in that area is equally distributed, thus causing the disappearance of any energy gradient, all flow of energy ceases. The plant in a field of energy, or in any considered area in which gradients exist, reacts passively to its surroundings. As will be demonstrated, the extent to which a plant can affect the amount of its heat exchange with its environment is quite limited; its possibilities of regulating the allotment of energy to the different forms of heat transfer are slightly greater.

As stated in the classical work of Brown & Escombe (10) and later in the comprehensive monographs of Büttner (13) and Huber (37), the heat transfer between the plant and its environment occurs essentially in three ways: through conduction and convection in the form of sensible heat; through the evaporation of water (to which we add the processes: condensation, freezing, thawing, and sublimation) in the form of latent heat; and by radiation.

The fact that heat transfer occurs not in one unique way but rather as two or more different physical events, leads to an interesting conclusion for the ecologist: The concept "environment" describes a space that is dependent upon the form of heat transfer being considered; differing forms of transfer take place in different spaces.

Hofmann (35, 36) and Raschke (65) have taken the concept "boundary layer" from technology and related it to the study of the heat transfer of plants. Since Prandtl (1904), this term has been used to describe a transfer zone that develops in a gas or liquid where that gas or liquid passes over a solid. The solid has no effect on the medium flowing outside of this zone; inside the zone, it influences the intermediate values of temperature, moisture content, and velocity [see e.g., (20, 70, 79)].

When the thickness of the boundary layer of leaves is calculated (65),

[1] The survey of literature pertaining to this review was concluded in July 1959.

values are obtained that are confirmed by measurements of the humidity gradient close to the leaf (62) and the temperature field around the leaf (7). According to these statements a leaf's exchange of moisture and temperature occurs in a zone whose thickness in extreme cases may exceed 1 cm. but is generally much smaller. In the case of strong wind and small leaves the width of this zone can be reduced to a fraction of a millimeter. The extent of the boundary layer is small, but the air in it is replaced constantly with the result that the amount of energy exchanged is more than the boundary layer width would seem to indicate possible; however, the sphere of influence of the plant is still astonishingly small. Only with a large group of plants can this influence reach decimeter or meter proportions. This discussion leads to questions of microclimatology which Geiger (25) treats.

By contrast, that part of the plant's environment taking part in radiation exchange is extremely large. The sun, as well as the ground and other reflecting surfaces, is part of the environment of visible radiation. Long-wave infrared radiation takes place in an even larger part of the environment. Like every other body, the plant emits radiation proportional to the fourth power of its absolute temperature and receives infrared radiation from all bodies that are visible from it. Admittedly, part of this radiation is absorbed by the intersticed air; but even the air exchanges radiation with the plant because CO_2 and water vapor absorb and emit infrared rays. Exchange of radiation with the air does not happen in a continuous spectrum but in bands separated by so-called windows. If the sky is clear, radiation is lost to space from these windows at 4 μ and between 9 and 11 μ wavelengths.

Environment depends on the wavelength of radiation: it may extend a few meters, into the higher atmosphere, or even into outer space. It is surprising that the botanist neglects the significance of the infrared radiation exchange; radiation laws have been known for 80 years and such contributions as Curtis' (18) provide the proper emphasis.

After what has been said it should be clear that the sun, or even outer space, is not more removed from the plant than the next millimeter of air; it is the specific part of heat exchange being considered that determines what should be included in the plant's environment. With an appropriate definition, it might be possible to describe the term "environment" in spatial terms, but to do so violates the unity of the heat exchange processes.

Components of Heat Transfer

Since the appearance of Huber's monograph (37) only a few works have been published that deal with heat transfer problems in botany. In some of these papers it was demonstrated that methods used in technology and microclimatology can be used in botany, a study lending itself more and more to treatment in physical rather than merely descriptive terms.

Conduction and convection.—If a temperature gradient exists between a plant and its environment and the resultant heat transfer occurs on the molecular level, this heat transfer is known as conduction. Convection in-

volves larger quantities of air or water. Heat conduction between the plant and the environment normally involves only the subterranean plant parts or those parts covered by snow [heat conductivity for soil: $\lambda = 0.09$ to 0.24; for snow: $\lambda = 0.01$ to 0.04 cal./cm.²/°C./min. (25)].

On the other hand, convection, which is more intense, is almost always effective between the plant and air (or water). The convective transfer is caused by the temperature difference $T_{Bl} - T_L$ in °C.[2] between plant and surrounding medium and is proportional to this difference. Therefore the flow of energy has to overcome the resistance of the boundary layer, whose property as a heat conductor is described by the heat-transfer coefficient h [cal./cm.²/min./°C.] (20, 27, 41); therefore $K = -h(T_{Bl} - T_L)$ [cal./cm.²/min.]. The dimension of h corresponds to Brown's & Escombe's "thermal emmissivity" and Huber's "Wärmeaustausch" (10, 37). Given the condition of a plant part in flowing air or water, the thickness of the boundary layer increases as a function of the distance from the point of the medium's initial contact with the plant part. As a result there is no uniform heat transfer coefficient. Each point on the surface of a plant has its own coefficient, which is admittedly one of a continuous series. Heat-transfer coefficients of a *Canna* leaf have been measured and presented topographically (65). As is to be expected, the values on the windward side and the border of the leaf were highest.

In further treatment it might be practical to proceed as in technology and assign to a body one mean, representative heat-transfer coefficient (35, 65). According to what has been said before, this mean value must decrease with increasing body size.

Comparisons have been made of the determinations of the heat-transfer coefficients of leaves and other similarly shaped bodies, and the diminishing effect of the wind on the boundary layer has been considered. Thus, the dependence of the heat-transfer coefficient on x (the width of the leaf) and on u (the wind velocity) is described satisfactorily by $h = (constant)\ (x^{-0.3})\ (u^{0.5})$. The constant indicates the influence of the shape of the leaf. When, for example, if one of two leaves that are similar

[2] All symbols in this paper are the same as those used in more detailed papers (65, 66). These symbols are: a factor, converting gradient of water vapor pressure into gradient of latent energy; B, storage of sensible heat; c, specific heat and g, weight of plant; c_P, specific heat of air as constant pressure; dE/dT, water vapor pressure change with temperature; dT/dt, temperature change with time; E_{Bl}, saturation water vapor pressure at plant temperature; E_L, saturation water vapor pressure at air temperature; ε, absorptivity; h, heat transfer coefficient; h_s, infrared radiation transfer coefficient; K, corrective transfer; P, exchange of chemical energy; r, relative humidity of the air expressed as a decimal fraction; R_{Bl}, resultant of diffusion resistances within the plant; R_G, diffusion resistance of the boundary layer; ϱ, density; S, radiation balance; S_L, radiation balance at air temperature; T_{Bl}, plant temperature; T_L, air temperature; θ, temperature difference between leaf and air; V, transfer of latent heat.

in form is only a fourth as wide as the other, then according to the formula the larger leaf must be exposed to a wind velocity 2.3 times higher in order to obtain the same heat transfer conditions as exist for the smaller. It should be added that only with the thinner leaves is the heat-transfer coefficient proportional to the square root of the wind velocity. Measurements of thicker plant parts might reveal a greater dependence on wind velocity (65).

Thus, "heat-transfer coefficient" has been used as a technological term and found very useful. For the sake of uniformity it is now replaced by "diffusion resistance R_G of the boundary layer", a term used in connection with transpiration and which is nothing but the reciprocal value of the heat-transfer coefficient multiplied by the density ρ and the specific heat c_p of the air at constant pressure: $R_G = c_p\rho/h$[cm.$^{-1}$/min.] (under normal conditions $c_p\rho = 2.88 \times 10^{-4}$).

Numerical values for heat-transfer coefficients and boundary layer resistances have been determined experimentally (4, 10, 11, 37, 55, 65), and theoretically (35, 65). According to these determinations the heat-transfer coefficients of a leaf 0.5 cm. wide increase from 0.03 to 0.3 cal./cm.^{2}/min./°C. when the wind increases from almost still air (0.1 m./sec.) to storm proportions (10 m./sec.). Under the same conditions the values for a leaf 10 cm. wide are 0.01 and 0.1 cal./cm.^{2}/min./°C. Expressed differently, this corresponds to boundary layer resistances from 0.01 to 0.001 cm.$^{-1}$ min. for the small leaf of the example and from 0.03 to 0.003 cm.$^{-1}$ min. for the larger. Determinations make it clear that the value of the coefficient for heat transfer by convection in open air can rarely be more than ten times the value found in the laboratory; greater values have been given (38), but these refer to meteorological "Austausch" and are not relevant. It should be pointed out that air in the laboratory is not absolutely still and usually fluctuates between 0.1 and 0.3 m./sec. This is expressed in corresponding fluctuations of the heat-transfer coefficient. Although heat-transfer coefficients for water plants are lacking, determinations are possible if technical literature is used (20, 28, 41).

Transfer of latent heat.—The temperature of the plant has a very essential influence on the water vapor exchange with the environment. It determines the saturation vapor pressure E_{Bl} of the moisture-emitting surface. The vapor pressure depression caused by dissolved substances can be ignored in the case of the plant (32). The gradient between E_{Bl} and the partial pressure e of the moisture in the air starts a water vapor stream. During this process the water changes its physical state and thus a transport of matter and a transport of energy are connected. As in convection the intensity of exchange depends on the resistance caused by the boundary layer: $V = -(E_{Bl} - e)\ h/a = -(E_{Bl} - e)c_p\rho/aR_G$ [cal./cm.^{2}/min.]. In this formula, a represents the simultaneous conversion of the vapor pressure gradient into a concentration gradient and the matter transport into a heat transfer. Under normal conditions a has the value of 0.5 (1, 65). Hofmann applies

a correction factor of the magnitude of 1.11 which takes into consideration the molecular transport of the boundary layer (35).

What has been said so far is only true of a body with a completely moist surface (e.g., an evaporimeter). In plants the resistance of the boundary layer is only one of the diffusion resistances along the moisture gradient (4, 24, 66, 71, 92, 93). These resistances can be considered joined in a fashion analogous to that in which electrical resistances are joined (66, 93). To simplify further treatment, all resistances with the exception of the boundary layer are represented by a resultant R_{Bl}. The flux of latent heat is then $V = -(E_{Bl} - e)c_p\varrho/a\ (R_{Bl} + R_G)$ [cal./cm.^{2}/min.] (66). The not entirely objectionless concept "Wasserbedeckungsfaktor" can be eliminated with the introduction of diffusion resistances (1, 13, 64, 65, 66).

To date, unfortunately, only a few statements including dimensions of the absolute size of the resistances within the plant are available. The few experimental determinations that have been made agree well with the detailed calculations of Bange (4, 66, 92). (It should be noted that Bange's conceptions "macro vapour cup" and "adhering air layer" correspond to the boundary layer.) The range of the resistance R_G of the boundary layer is 0.001 to 0.03 cm.$_{-1}$ min. The range of the resistance R_{Bl} within the leaf however, according to experimental results, is between 0.005 and 3.0. In the calculation of resistances of stomata an even greater range, 0.0004 to 4.0 cm.$^{-1}$ min., is to be expected (66). Measurements have confirmed that in closed stomata the wind has practically no effect on transpiration (75, 78). If, for example, R_{Bl} is assumed to be 0.5 cm.$^{-1}$ min., the maximum possible change of the total resistance $(R_{Bl} + R_G)$ is 0.501 to 0.53.

The plant exchanges latent heat not only by transpiration but also obtains energy from dew, hoarfrost, and rime and loses it again by sublimation and evaporation in the case of a wet surface (34, 35, 36). These processes are influenced only by the resistance of the boundary layer (i.e., by wind velocity and organ size). Finally, a wet plant can gain heat when water freezes. This process is applied practically in anti-frost sprinkling [see (68, 94, 95) for more literature].

Radiation.—A current, comprehensive study by Härtel & Sauberer of the plant and radiation gives information about the intensities of natural radiation, the influence of the exposure of the plant, and the radiation properties of plant parts in the different wavelength ranges (28).

Of the total radiation received by the plant, only a part [44 to 88 per cent (65)] is absorbed and part of this is emitted again in the long-wave region (with a maximum at wave lengths between 8.9 and 10.5 μ).

The remaining radiation balance has been measured in a very few cases (64). The experimental results for plant covered areas are more numerous [(5, 6, 22, 23, 54, 74) summary in (25)]. Usually the net radiation must be estimated by balancing the radiation components (65). Newly published material on the radiation reception of inclined surfaces should be useful for

estimating the radiation balance. Some such published matter exists. The paper of Kondrat'ev & Manolova is especially notable because of the inclusion of diffuse sky radiation (45, 60, 61).

In the region of long-wave infrared radiation the plant is almost a black body [$\varepsilon \geqslant 0.95$ (25, 28)]. The net loss of infrared radiation because of higher temperature is generally greater during the day than night and may reach a rate of loss of 0.2 cal./cm.^{2}/min., a rate which may be even higher in a hot climate (64).

Interdependence of Components

The plant is practically never in thermodynamic equilibrium with its environment. Because the temperature of the sun is thousands of degrees higher than the temperature of the plant, energy is radiated to the plant and there transformed into heat. This process causes new gradients. Even if no visible radiation is present, generally the temperature of part of the environment (at least parts of the atmosphere) differs from that of the plant, causing a long-wave radiation exchange. Also, vapor pressure differences between the plant and the environment create new gradients. Energy conversions occur concurrently during every heat exchange between the plant and environment, thus making inadequate a quantitative treatment of the dynamics of a single, isolated component. A treatment must consider the totality of the energy economy.

Equation of energy balance.—Heat economy equations have thus far been given for plant surfaces (e.g., 1, 12, 25, 28, 36, 54) and only in the case of thin leaves for bodies (e.g., 1, 10, 37, 43, 64, 65, 89). At any instant, the intake and the expenditure of the heat-transfer processes, the chemical reactions P, and the warming or cooling $cgdT/dt = B$ of the plant (37) must balance: $O = K + V + S + P + B$. Since photosynthesis normally rarely uses up more than 2 per cent of the absorbed, photosynthetically-active radiation [(91), higher values in (57)], and since only in exceptional cases does respiration result in measurable temperature effects (37 to 39, 80), P can be disregarded. Though the storage and conduction of heat in tree trunks (44) and succulents (33, 37, 88) causes a lag in thermal changes, this fact is unimportant for ordinary leaves (16, 37, 64, 72). So far, quantitative studies of heat economy have been conducted only on leaves, therefore B can be disregarded. These omissions simplify the physical treatment considerably, since now the processes can be considered steady.

In the future, admittedly, such a treatment could be unsatisfactory; the treatment of these processes as functions of time is a task needing much research. Researchers to date have contented themselves with the following, simplified heat balance equation as a basis for studying the energy exchanges: $O = K + V + S$. The balance terms are positive if energy flows toward the plant (35, 65).

Plant temperature: the crux of all heat exchange.—The magnitude and direction of heat exchanges by convection and radiation, and phase-changes

of water are determined by the plant temperature; heat exchanges determine temperature. The plant temperature states the potential at which the heat exchange processes are in equilibrium. This equilibrium is analogous to the water level in a vessel possessing several inlets and outlets. The water level depends not on the inlet and outlet sizes but rather on the relationship between the two. Thus the plant temperature reveals nothing about the magnitude of the energy exchange, a fact contrary to the belief of those who confuse temperature with amount of heat.

Because the plant temperature is of key significance, knowledge of it is important particularly in extreme conditions; however, the environmental conditions in which these temperatures occur must also be known. Some of the works that appeared after Huber's last summarizing paper (38) about the temperature of plant surfaces should be mentioned. Warren Wilson, in a comprehensive study, measured and explained previously cursorily described conditions in the Arctic (90). Henrici (30), Lange (51), and Vaupel (88) performed measurements in hot, dry regions. *Pinus cembra* needles are exposed to large thermal stresses on the alpine timber line [Tranquillini (85); Turner (86)]. In Tranquillini's paper statements are found about the temperature of needles under snow, where, as also stated by Kil'djuševskij, the temperature seems never to drop below −5°C. (42). Large daily ranges in dry lichens and mosses were measured by Lange (50). Tree trunks exhibit a relatively moderate temperature curve [Bergström (8); Koljo (44); Kunii (48)] and here the heat conduction properties of the bark play a role [Shcherbakov (73)]. Like plants in the open air, the temperature of submerged water plants can also deviate from that of the environment [Casperson (14); Schanderl (69)]. When taking measurements it should not be forgotten that a plant part does not have to be of uniform temperature. Raschke measured temperatures across a leaf, and Casperson the temperature differences between the top and the bottom of a leaf [(16, 65); see also Vaupel's data (88)].

The plant temperature influences each of the heat transfer proceses in a different way. Convection and conduction are proportional to the temperature difference between plant and environment. The radiation loss in infrared increases with the fourth power of the absolute temperature and the saturation vapor pressure of water follows approximately an exponential function. Because of this the heat economy equation is transcendental and cannot be solved as it is. One must deviate from the classical approach in order to describe the quantitative interdependence, of which the plant temperature is a statement, of the heat transfer components. Thus far only procedures used in the case of the leaf have been given.

Solution of the equation of balance.—One can rearrange the heat economy equation in such a way as to obtain a linear function on one side and a vapor pressure function on the other. If both functions are portrayed graphically, their point of intersection gives the temperature of the leaf (65, 66). This graphical solution is exact, but time consuming. The goal can

be reached more quickly and with satisfying accuracy by a process of mathematical approximation [Hofmann (34, 35, 36); Raschke (65)]. Here one considers only the temperature difference between leaf and air ($\theta = T_{Bl} - T_L$). Further, in applying this method one assumes that the curves of the radiation and vapor pressure functions considered within a small range can be replaced by their tangents at the point T_L (differential quotient replaces "difference" quotient). If, in a presentation, the influence of the diffusion resistances is expressed as in (66), for convection $K = -\theta c_P \rho / R_G$ is obtained. The latent heat transfer is represented by $V = -[E_L (1 - r) + \theta \, dE/dT] \, c_P \rho / (R_G + R_{Bl})$. ($E_L$ stands for the saturation vapor pressure of the water at air temperature and r for the relative air humidity). In the presentation of the radiation balance one assumes first that the leaf has the same temperature as the air (S_L represents this) and then corrects the value corresponding to the temperature difference θ between leaf and air: $S = S_L - 2\,\theta h_s$, wherein h_s is a radiation transfer coefficient depending on T_L (35, 65).

Now the equation can be solved. One obtains $\theta = [S_L - nE_L (1 - r)]/[2 \, (h + h_s) + ndE/dT]$ with $n = c_P \rho / a(R_G + R_{Bl})$.[3] Thus the temperature difference between leaf and air consists, as has long been known, of a radiation term and an evaporation (or condensation) term with both terms independent of each other. Consequently the heat exchange by convection must consist of two corresponding members. The relationship between latent exchange on the one hand and radiation and convection on the other can now be expressed in the following formula: $V = -mS_L - n (1 - m) \, E_L \, (1 - r)$ where $m = 1/[2a \, (R_G + R_{Bl}) \, (c_P \rho / R_G + h_s)/(c_P \rho dE/dT) + 1]$. The top and the bottom surfaces of the leaf may be considered simultaneously and separately with this method. It can be concluded from the formula that the latent heat exchange consists of a radiation term and a humidity term which are independent of each other (35, 65, 66). According to the heat economy equation, the radiation balance must also divide itself into two streams, one of which feeds the radiation term of latent exchange, and the other, the radiation term of convection.

Summarizing, one comes to the conclusion that each of the three most important heat transfer processes consists of complements which might quite possibly be of different signs. Simultaneously, it becomes clear that each process is limited by the amount of energy available. Thus, transpiration is not only a process of diffusion, but is also one of heat loss that can never exceed the magnitude of the energy gained by radiation and convection (36, 65). Calorimetric methods for the measurement of transpiration are based on this relation (11, 63).

Dependence of Components on Plant

The effects of plant and environment on the heat transfer in which both

[3] All values calculated in this manner are mean values for the surface considered. The local values can deviate from the mean value considerably (65).

are involved are interwoven to such an extent that they should be described together. If they are separated despite this, it is for the sake of clarity; however, their relationship is the background of this treatment.

Morphological and anatomical properties of a plant may influence the volume of the total heat exchange as well as the predominance of this or that heat-transfer process. The statement of such a relationship does not indicate teleological thinking.

Radiation.—Even during the night, the amount of total energy turnover is almost always determined by the radiation balance that, in turn, depends on the absorptivities and position of the organ considered. The absorptivity can change only a little (e.g., by movement of chloroplasts); yet it is quite possible that a leaf can lean toward radiation, which is important in the Arctic (90), or in other cases to the profile position, thus reducing energy reception to a minimum [(37), other possible effects of organ position given by Vaupel (88)]. This leads to the problem of compass plants, for which it has not been proved that the radiation field alone affects their direction (21). If radiation balance changes with leaf position, so do the radiation terms of transpiration and convection. Corresponding measurements of temperature and transpiration have been given by Konis (46). Prickles and hairs seem to lower radiation absorption only slightly; they affect the convection exchange with the environment to an equally small extent, so they have been termed "thermisch neutral" (2, 31).

Resistance of boundary layer.—The morphology of the plant influences the formation of the boundary layer and in this way especially affects the heat transfer by convection, but it also affects transfer through latent heat. Thus, a very small leaf or a compound leaf with small leaflets has a very thin boundary layer in which a small temperature deviation suffices to bring about an effective convection transfer (37, 65, 76). Small and doubly-compound leaves are found especially in hot climates rich in radiation (67, 81). The low boundary layer results simultaneously, it is true, in a large humidity term of transpiration; but the plant, as already mentioned, is in a position to put very high diffusion resistances into effect and thus budget the water (65, 66). In addition, high heat-transfer coefficients help reduce the radiation term of transpiration, as is confirmed by the findings of Mitchell & Closs (53). They observed that long grass loses less water than short grass in summer; in spring, long grass loses more.

The formation of a thick boundary layer, on the other hand, affects temperature differences from the air that are correspondingly large. So it happens, sometimes, that in nocturnal radiation loss only large leaves lose enough heat to cool below the dew point of the air. Then dew condenses on their surfaces while small leaves remain dry. Since boundary layer thickness increases with distance from the leaf margin, often only the middle of the leaves are dew bedecked (35, 65). These are also the areas that freeze first in radiation frost (87).

Recently a contradictory observation has been reported according to which dew and hoarfrost are predominantly formed on the leaf margins (77).

Physically this is impossible if the cooling is caused by radiation loss. The observations leading to this conclusion could have been caused by a deposition of water from an oversaturated atmosphere. As Hofmann (35) demonstrated, in such a case water crystallizes in the form of rime on those areas where the boundary layer is thinnest. Another example of the existence of large temperature deviations from the air temperature is the cushion and dwarf growths. The wind, which has already been weakened by the proximity of the soil surface, is still more reduced by the thick felt of the plant; thus radiation can result in a strong warming effect. The temperature near the ground, which in any case is higher during the day, serves in this instance as a reference. Plants, then, can become photosynthetically active despite low air temperatures measured two meters above the ground [Bliss (9); Hirsch (33); Warren Wilson (90)]. In the Arctic, pussy willows reach a temperature in the sun that averages 20°C. above air temperature. Radiation is absorbed by a dark interior layer, and an enclosing layer of glossy hair offers a very high resistance to heat transfer by convection [Krog (47)]. It should be mentioned that possibly the formation of rosettes should also be considered as a form of growth that influences heat transfer. Kunkel states that the middle of the *Echinum* rosette was 20°C. cooler in the sun than the nonvegetated ground surface, and therefore it protects the shoot and root axes from overheating (49).

Internal resistances.—The influence of the plant on the distribution of the received radiation energy is accomplished by diffusion resistances located in the mesophyll membranes, the intercellular spaces, the stomata, and the cuticle, and which are all represented by the symbol R_{Bl}. If R_{Bl} increases, less heat is dissipated by transpiration and more by convection, and the temperature of the plant rises. It is on this level that the water economy of the plant affects the heat transfer processes.

Another effect ascribed to internal resistances is that a leaf reacts differently from a wet body, as shown by measurements [e.g., (3)], even if the resistances remain constant. As has already been explained, fluctuations in the resistance of the boundary layer caused by the wind have an unhindered and full effect on evaporation; in the leaf the size of this effect depends upon what part the boundary layer resistance is of the total resistance. In its physical behavior the leaf lies between the reactions of a wet body and a corresponding dry body of the same size, shape, and radiation absorptivities. The location of the leaf reaction is stated by $R_G/(R_G + R_{Bl})$, a ratio formerly inaccurately expressed by the "Wasserbedeckungsfaktor" (66). In a perfect wet body the radiation term for evaporation is independent of the wind; the humidity term, on the other hand, increases as the boundary layer decreases, mostly with the square root of wind velocity (65). If another diffusion resistance R_{Bl} is added, both terms become smaller, and simultaneously their dependence on the boundary layer resistance shifts; with increasing R_{Bl} the humidity term becomes almost constant, while the radiation term is increasingly more influenced by the wind in such a way

that less transpiration is caused by a stronger wind (66). The temperatures of the evaporimeter and the leaf depend differently on increasing wind speed. While the evaporimeter approaches the wet bulb temperature of a psychrometer, the leaf temperature approximates asymptotically the air temperature (65, 66).

Dependence of Components on Environment

In this section we will confine ourselves chiefly to a treatment of latent heat transfer because, if the radiation balance is known, convection, the single unknown variable remaining, can be easily found. In addition the six terms of the heat transfer components are arranged in pairs, the members of which have equal magnitudes and opposite signs: the humidity term of latent transfer and the latent transfer term of convection, the radiation term of latent transfer and the latent transfer term of radiation, and the convection term of radiation and the radiation term of convection.

Besides the environmental factors of radiation, humidity, and wind, which will shortly be considered in detail, the air temperature T_L and the air pressure influence the heat-transfer processes. Air temperature has a threefold effect. First, it is the reference on which plant temperature is based. Second, it states the level at which the latent transfer occurs. Because dE/dT depends heavily on temperature and is a factor in the radiation term of transpiration, the efficiency of radiation as a cause of transpiration increases with increasing temperature (36). Third, the radiation transfer coefficient h_s increases with temperature [data in (35, 36, 65)]. The influence of the air pressure works through the factor a and is significant only at high altitudes. Hirsch's observation of high water losses despite the low saturation deficit in high mountains could be explained in this manner (33).

Radiation.—Even if the air is saturated, plants can still transpire if received radiation must be dissipated. In this case the radiation term of transpiration accounts for the total latent transfer. Under natural conditions in such a case water immediately condenses in the air. This explains the "smoking" of the forest after rain. But radiation usually remains the chief source of energy, even if the air is not saturated. Therefore it is no surprise if transpiration follows the "diurnal march" of radiation during the day. This dependence was measured by Raschke (64) on a single leaf and by Nutman on *Coffea* and *Eugenia* trees (58, 59). Nutman ascribes this to stomata reactions, but it could be explained simply as the necessary result of energy budgeting. Stocker's explanation is similar to this latter conclusion (78). An especially strong relationship between radiation and transpiration exists in glass houses (56) and in the tropics where the humidity term in transpiration is rather small because of the low saturation deficit. Thus it is not surprising that 90 per cent of the monthly variation in evaporation from a sugarcane field is to be attributed to radiation (17). But even in temperate climates (which have an absolute saturation deficit of about the same size as that of the tropics) water losses from plant-

covered surfaces are closely correlated with radiation balance, a fact noted by Frankenberger (22, 23). Correlation coefficients calculated from these data by Michalowsky vary between 0.76 and 0.95 (52). It is interesting that Frankenberger derives an empirical evaporation formula which virtually coincides with that obtained by mathematical approximation, a fact pointed out by Hofmann (36). Ivanov & Silina were encouraged by the relationship between radiation and transpiration to make actinometric measurements of transpiration from forests (40).

Long-wave radiation plays a considerable role in radiation exchange; e.g., any change of soil-surface temperature is reflected in a change of the energy budget of the leaf exposed above. An *Alocasia* leaf received 12 per cent less net radiation above irrigated soil than above a dry, hot surface [(64); more examples in (25)]. Long-wave radiation losses to atmosphere and space are mostly responsible for nocturnal frost damage in spring or fall. Such damage can be checked by covering the crops with mats or foils, by artificially increasing the turbidity of the air ["closing the windows," theoretical treatment in (19)], by wind machines (removing temperature stratification near the ground), and by orchard heaters, which are also a source of radiation [summary in (25)].

When water is sprinkled as an anti-frost measure (68, 94, 95), heat liberated by the water freezing on the plant has to compensate energy lost by radiation, convection, and evaporation. Dew and hoarfrost as results of long-wave radiation losses and as influenced by humidity and wind have been dealt with by Hofmann (34 to 36).

Sometimes absence of an effective long-wave radiation escape may lead to severe overheating of the plant, especially in glass houses, because glass is opaque to infrared. Stagnant air and high humidity hinder dissipation by convection and transpiration, and thus cause a thermal load that may be greater than the thermal load in the tropics. Merely slight ventilation might be beneficial in such a case. Similar conditions prevail in "cuvettes," which are used for the study of gas exchange (83 to 85).

Saturation deficit.—From what has been said it appears that the humidity of the air is only of minor importance. This idea may have caused Thornthwaite to ascribe to the "vapor pressure deficit very little value as an indicator of moisture losses" (82). This is somewhat infelicitous according to the other works referred to in this review (36). When net radiation is zero, the heat budget is proportional to the saturation deficit [e.g., (26)]. Determinations in a temperate zone have indicated that the humidity term is somewhat less than 25 per cent of the total water losses (36). As radiation decreases, the humidity term becomes more prominent; this is proved by the excellent observations of Nutman (58, 59). Further proof is supplied by the observations of Halevy (29). He found that transpiration differences between sun and shade leaves are smaller than usual on "sharav" days, which are very dry and low in radiation. On these days the humidity terms, which are the same for both types of leaves, are more important

than the radiation terms. A remark of Clum [cited in (3)] is noteworthy in this connection. He states that in Arizona and Kansas leaf temperatures are near or slightly above air temperature while in the New York region they are normally several degrees above air temperature. One can assume that in drier states more energy is lost by transpiration than in New York, because of the high humidity there, and thus in New York more heat must be dissipated by convection.

Wind.—As already shown previously, the effects of boundary layer resistance—this means also the effects of wind—on the heat-transfer components are considerably affected by the internal resistances R_{Bl} of the plant. The humidity term in transpiration increases with wind, or, if R_{Bl} is high, remains constant; the radiation term is constant, or, if R_{Bl} is high, decreases. Thus, depending on prevailing stomata width, radiation, and humidity, total transpiration will increase, decrease, or not react at all (66). It can be concluded from this that past findings whose variances produced controversy could all have been correct. It can also be concluded that wind sheltering may have different values. Behind a wind break, heat-transfer coefficients are smaller, and thus temperature differences between plant and air must be larger if gains and losses are to be balanced by convection [see measurements by Casperson (15, 16)]. This effect may be beneficial in latitudes where temperatures are below optimum (90). In hot climates and under strong insolation, however, wind sheltering could be harmful to the plant, especially when a reduction of wind results in an increase of the radiation term of transpiration; shading avoids this and therefore is widely used.

Determination of Heat Transfer Between Plant and Environment

Calculations and recordings of the energy exchange of the leaf, as well as other experimental data quoted in this review, do not suffice as yet as a proof that the relations and the values obtained by using physical models are representative. Measurements, which are still needed, will be difficult to perform. Further, a quantitative treatment of the plant's reactions as well as a theoretical approach to nonstationary processes will be even more difficult.

Since this review was restricted to a treatment of the individual plant, the author reluctantly had to refrain from citing valuable studies of the energy budget of plant-covered surfaces, which were performed chiefly by meteorologists. A summary of these studies is presented in Geiger's book (25).

LITERATURE CITED

1. Albrecht, F., *Arch. Meteorol., Geophys. u. Bioklimatol. Ser. B,* **2,** 1–38 (1950)
2. Alsaç, N., *Beih. z. Botan. Centr.* **A 61,** 329–68 (1942)
3. Arthur, J. M., and Stewart, W. D., *Contribs. Boyce Thompson Inst.,* **5,** 483–501 (1933)
4. Bange, G. G. J., *Acta Botan. Neerl.,* **2,** 255-97 (1953)
5. Baumgartner, A., *Forstwiss. Centr.,* **75,** 223–39 (1956)
6. Baumgartner, A., *Untersuchungen über den Wärme- und Wasserhaushalt eines jungen Waldes* (Ber. d. Deut Wetterdienstes, **5,** Nr. 28, Bad Kissingen, Germany, 51 pp., 1956)
7. Berger-Landefeldt, U., *Ber. deut. Botan. Ges.,* **71,** 21–33 (1958)
8. Bergström, H., *Svensk Papperstidn.,* **61,** 902 (1958)
9. Bliss, L. C., *Ecol. Monographs,* **26,** 303–37 (1956)
10. Brown, H. T., and Escombe, F., *Proc. Roy. Soc. (London) B,* **76,** 29–111 (1905)
11. Brown, H. T., and Wilson, W. E., *Proc. Roy. Soc. (London) B.,* **76,** 122–37 (1905)
12. Budagowski, A. I., and Sawina, S. S., *Meteorologiia i Gidrologiia,* No. **8,** 20–23 (1956)
13. Büttner, K., *Die Übertragung durch Leitung und Konvektion, Verdunstung und Strahlung in Bioklimatologie und Meteorologie* (Abh. preuss. Meteorol. Institut., **10,** Nr. 5, 37 pp., 1934)
14. Casperson, G., *Ber. deut. botan. Ges.,* **69,** 479–86 (1956)
15. Casperson, G., *Angew. Meteorol.,* **2,** 339–51 (1957)
16. Casperson, G., *Z. Botan.,* **45,** 433–73 (1957)
17. Cowan, I. R., and Innes, R. F., *Proc. Brit. West Indies Sugar Technologists, 1955*
18. Curtis, O. F., *Plant Physiol.,* **11,** 343–64 (1936)
19. Drimmel, J., *Arch. Meteorol., Geophys. u. Bioklimatol., Ser. B,* **6,** 443–51 (1955)
20. Eckert, E., *Einführung in den Wärme- und Stoffaustausch* (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1949)
21. Falk, H., *Beitr. Biol. Pflanz.,* **32,** 457–69 (1956)
22. Frankenberger, E., *Ann. Meteorol.,* **7,** 47–52 (1955)
23. Frankenberger, E., *Meteorol. Rundschau,* **7,** 81–5 (1955)
24. Füsser, K., *Planta,* **19,** 485–533 (1933)
25. Geiger, R., *Das Klima der bodennahen Luftschicht,* 4th German ed. (Friedr. Vieweg & Sohn, Braunschweig, to be published early in 1960)
26. Griep, W., *Z. Botan.,* **36,** 1–54 (1940)
27. Gröber, H., and Erk, S., *Die Grundgesetze der Wärmeübertragung,* 3rd rev. ed. (Grigull, U., Ed., Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 428 pp., 1955)
28. Härtel, O., and Sauberer, F., *Pflanze und Strahlung* (Akad. Verlagsgesellsch., Geest & Portig K.-G., Leipzig, E. Germany, 1959)
29. Halevy, A., *Bull. Research Council Israel, Sect. D,* **5,** 165–75 (1956)
30. Henrici, M., *S. African J. Sci.,* **51,** 245–48 (1955)
31. Herzog, F., *Jahrb. wiss. Botan.,* **87,** 211–43 (1938)
32. Hertel, W., *Flora,* **133,** 143–214 (1938)
33. Hirsch, G., *Beitr. Biol. Pflanz.,* **33,** 371–422 (1957)

34. Hofmann, G., *Ber. deut. Wetterdienstes, U. S. Zone,* **38,** 360–64 (1952)
35. Hofmann, G., *Die Thermodynamik der Taubildung* (Ber. d. Deut. Wetterdienstes, **3,** Nr. 18, Bad Kissingen, Germany, 45 pp., 1955)
36. Hofmann, G., *Planta,* **47,** 303–22 (1956)
37. Huber, B., *Der Wärmehaushalt der Pflanzen* (Verlag Dr. F. P. Datterer & Cie., Freising-München, Germany, 148 pp., 1935)
38. Huber, B., *Handbuch der Pflanzenphysiologie,* **3,** 285–92 (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1956)
39. Hummel, K., *Meteorol. Rundschau,* **1,** 147–50 (1947)
40. Ivanov, L. A., and Silina, A. A., *Fiziol. Rastenii, Akad. Nauk. S.S.S.R.,* **2,** 313–19 (1955)
41. Jakob, M., and Hawkins, G. A., *Elements of Heat Transfer,* 3rd ed. (John Wiley & Sons, Inc., New York, N.Y.; Chapman & Hall Ltd., London, England, 1957)
42. Kil'djuševskij, I. D., *Botan. Zhur.,* **41,** 1641–46 (1956)
43. Kleshnin, A. F., Strogonov, B. P., and Shulgin, I. A., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.,* **2,** 549–57 (1955)
44. Koljo, B., *Forstwiss, Centr.,* **69,** 538–51 (1950)
45. Kondrat'ev, K. Ia., and Manolova, M. D., *Meteorologiia i Gidrologiia,* No. 6, 31–4 (1955)
46. Konis, E., *Ecology,* **31,** 147–48 (1950)
47. Krog, J., *Physiol. Plantarum,* **8,** 836–39 (1955)
48. Kunii, K., *J. Meteorol. Research (Tokyo),* **4,** 1035–38 (1953)
49. Kunkel, G., *Biol. Zentr.,* **77,** 278–300 (1958)
50. Lange, O. L., *Arch. Meteorol., Geophys. u. Bioklimatol., Ser. B,* **5,** 182–89 (1954)
51. Lange, O. L., *Ber. deut. botan. Ges.,* **70,** 31–2 (1958)
52. Michalowsky, J., *Ann. Meteorol.,* **8,** 7–10 (1957)
53. Mitchell, K. J., and Closs, R. L., *Nature,* **181,** 1619–20 (1958)
54. Muminov, F. A., *Meteorol. i Gidrologijia,* No. 8, 36–40 (1958)
55. Nakagama, Y., *J. Agr. Meteorol.,* **13,** 17–21 (1957)
56. Neale, F. E., *Neth. J. Agr. Sci.,* **4,** 48–56 (1956)
57. Nichiporovich, A. A., and Chmora, S. N., *Fiziol. Rastenii, Akad. Nauk. S.S.S.R.,* **5,** 320–8 (1958)
58. Nutman, F. J., *Ann. Botany (London),* **5,** 59–82 (1941)
59. Nutman, F. J., *Ann. Botany (London),* **17,** 611–14 (1953)
60. Okanoue, M., *J. Japan. Forestry Soc.,* **39,** 435–37 (1957)
61. Okanoue, M., and Makiyama, M., *J. Japan. Forestry Soc.,* **40,** 40–1 (1958)
62. Ramsay, J. A., Butler, C. G., and Sang, J. H., *J. Exptl. Biol.,* **15,** 255–65 (1938)
63. Raschke, K., *Naturwissenschaften,* **41,** 308 (1954)
64. Raschke, K., *Arch. Meteorol., Geophys. u. Bioklimatol., Ser. B,* **7,** 240–68 (1956)
65. Raschke, K., *Planta,* **48,** 200–38 (1956)
66. Raschke, K., *Flora,* **146,** 546–78 (1958)
67. Richards, P. W., *The Tropical Rain Forest* (2nd ed., Cambridge University Press, England, 450 pp., 1957)
68. Rogers, W. S., Modlibowska, I., Ruxton, J. P., and Slater, C. H. W., *J. Hort. Sci.,* **29,** 126–41 (1954)
69. Schanderl, H., *Ber. deut. botan. Ges.,* **68,** 28–34 (1955)

70. Schlichting, H., *Grenzschicht-Theorie* (3rd ed., Verlag G. Braun, Karlsruhe, Germany, 608 pp., 1958)
71. Seybold, A., *Planta,* **9,** 270–314 (1929)
72. Seybold, A., and Brambring, F., *Planta,* **20,** 201–29 (1933)
73. Shcherbakov, I. N., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.,* **2,** 392–96 (1955)
74. Shul'gin, A. M., *Meteorol. i Gidrologiia,* No. 2, 32–34 (1956)
75. Stålfelt, M. G., *Handbuch der Pflanzenphysiologie,* **3,** 351–426 (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1956)
76. Stålfelt, M. G., *Handbuch der Pflanzenphysiologie,* **3,** 324–41 (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1956)
77. Steubing, L., and Casperson, G., *Z. angew. Meteorol.,* **3,** 219–24 (1959)
78. Stocker, O., *Handbuch der Pflanzenphysiologie,* **3,** 436–88 (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1956)
79. Sutton, O. G., *Micrometeorology* (McGraw-Hill, New York, N.Y., Toronto-Canada, and London, England, 333 pp., 1953)
80. Suzuki, S., and Yoshida, Y., *J. Agr. Meteorol.,* **6,** 1–4 (1951)
81. Talbert, Ch. M., and Holch, A. E., *Ecology,* **38,** 655–58 (1957)
82. Thornthwaite, C. W., *Handbuch der Pflanzenphysiologie,* **3,** 257–64 (Springer-Verlag, Berlin-Göttingen-Heidelberg, Germany, 1956)
83. Tranquillini, W., *Ber. deut. botan. Ges.,* **67,** 191–204 (1954)
84. Tranquillini, W., *Planta,* **46,** 154–78 (1955)
85. Tranquillini, W., *Planta,* **49,** 612–61 (1957)
86. Turner, H., *Arch. Meteorol., Geophys. u. Bioklimatol., Ser. B,* **8,** 273–325 (1958)
87. Ullrich, H., and Mäde, A., *Planta,* **28,** 344–51 (1938)
88. Vaupel, A., *Flora,* **145,** 497–541 (1958)
89. Waggoner, P. E., and Shaw, R. H., *Plant Physiol.,* **27,** 710–24 (1952)
90. Warren Wilson, J. W., *J. Ecol.,* **45,** 499–531 (1957)
91. Wassink, E. C., *Plant Physiol.,* **34,** 356–61 (1959)
92. Welten, M., *Planta,* **20,** 45–165 (1933)
93. Williams, W. T., and Amer, F. A., *J. Exptl. Botany,* **8,** 1–19 (1957)
94. Witte, K., and von Pogrell, H., *Rhein. Monatsschr. f. Gemüse-, Obst-u. Gartenbau,* **46,** 195–97, 267 (1958)
95. Witte, K., *Weinberg u. Keller,* **6,** 95–100 (1959)

THE RELATIONSHIP BETWEEN SALT UPTAKE AND THE ABSORPTION OF WATER BY INTACT PLANTS[1]

By R. Scott Russell and D. A. Barber

Agricultural Research Council Radiobiological Laboratory, Letcombe Regis, Wantage, Berkshire, England

Introduction

It is well established that the rate at which ions are absorbed by the roots of intact plants and transferred to their leaves may be considerably affected by the rate of concurrent transpiration [Wright (1); Phillis & Mason (2); Broyer & Hoagland (3); Michael & Wilberg (4); Brouwer (5, 6); Hylmö (7, 8, 9); Kylin & Hylmö (10); Russell & Shorrocks (11, 12)]. This has not been observed in all investigations however, and two conflicting views are currently held regarding the mechanism whereby transpiration can affect the overall process by which ions pass upwards through intact plants.

Some investigators claim that the transfer of ions across the root symplast to the vascular stele is an active process dependent on the release of energy through metabolic processes and that the effect of transpiration is to accelerate the movement of ions in water after they have been released into the vascular tissues [van den Honert *et al.* (13); Arisz (14); Broyer & Hoagland (3); Brouwer (5, 6); Russell & Shorrocks (11, 12)]. The opposing concept, that ions move passively in water from the outer surface of the root upward to the shoot, has lately received considerable attention [Michael & Wilberg (4); Hylmö (7, 8, 9); Epstein (15, 16); Kramer (17); Kylin & Hylmö (10)]. The primary object of a review of the relationship between salt accumulation and the absorption of water must therefore be to assess the relative merits of these opposed hypotheses.

Evidence That Ions Are Transferred Across the Roots of Plants by an Active Process

The investigations of Hoagland, Broyer, Steward, and their collaborators nearly three decades ago [Steward (18); Hoagland & Broyer (19)] and many subsequent workers [Robertson (20); Lundegårdh (21); Laties (22)] established that the accumulation of electrolytes in the vacuoles of plant cells depends upon the expenditure of energy derived from respiration. The existence of a high resistance barrier, now commonly regarded as the tonoplast, was clearly demonstrated by the presence of considerably higher concentrations of electrolytes within the vacuoles of cells than in the ambient medium.

[1] The survey of literature pertaining to this review was concluded in September 1959.

The majority of the detailed studies of the mechanism of salt accumulation have been carried out using relatively simple tissue systems such as slices of storage tissues, algae, or the detached roots of plants. None the less, many observations suggest that the process whereby ions cross the roots of intact plants also depends upon the expenditure of metabolic energy. The transfer of nutrients to shoots is affected by both temperature and the supply of oxygen in much the same way as is respiration [Broyer & Hoagland (3); Shapiro *et al.* (23)]. The dependence on respiration of the mechanism whereby ions are transferred across roots has also been indicated by the inhibitory effects of such substances as sodium azide, dinitrophenol, diethyldithiocarbamate, and cyanide [Lundegårdh (24); Robertson & Turner (25); Robertson *et al.* (26); Brouwer (5)] except under special circumstances [Russell *et al.* (27)].

Particularly clear evidence that energy must be expended in the transfer of ions across roots, and, furthermore, that a barrier to free movement exists, has been provided by experiments in which the concentration of ions in the vascular sap has been shown to exceed the concentration in the outer medium. Working with decapitated wheat roots immersed in solutions containing 1 m.eq. per l. of calcium nitrate, potassium nitrate, or potassium chloride, Lundegårdh (24) found that the concentration in the bleeding sap could exceed that in the outer medium by a factor of between 10 and 15. In experiments with decapitated barley roots Broyer (28) showed that the concentration of bromide in the exudate could reach some 30 times that in the external medium. Further evidence of the existence of a barrier to the free diffusion of ions across roots is provided by Lundegårdh's (29) very extensive observations on the relationship between the external concentration and the rate of bleeding of decapitated roots; an osmotic effect was clearly demonstrated which could not have occurred if ions were capable of free diffusion.

Numerous studies of the relative rates at which different ions are transferred across the cytoplasm of individual cells to the vacuole or across the symplast to the stele of an intact root have suggested the similarity of these two mechanisms; in general monovalent cations are absorbed considerably more rapidly than divalent cations [Hoagland (30); Collander (31)]. The presence of some barrier which impedes the passage of ions by mass flow in water is further suggested by the very slow rate at which polyvalent ions reach the aerial tissues of plants [Jacobson & Overstreet (32); Rediske *et al.* (33)].

Widely divergent views have been expressed about the mechanism whereby ions are transferred across roots. In 1920 Priestley (34) suggested that ions could freely pass within cell walls across the cortex to the endodermis, which he regarded as a barrier to further free diffusion. He concluded that ions were transported through the endodermal protoplasts and plasmadesmata into the stele. Priestley's views on the first stage of the passage of ions across the roots are strikingly similar to recent suggestions

based on the concept of "apparent free space." These are discussed later in this review. In 1938 Crafts & Broyer (35) concluded that ions pass across the cortical tissues of roots mainly in the cytoplasm; like Priestley, they regarded the endodermis as a barrier to free ionic movement. The symplast theory advanced later by Arisz (14) has a number of features in common with this view. He considered that ions entered the cytoplasm by an active process and then moved passively through the symplast until a final active process released them into the xylem. Lundegårdh (36) interpreted the results of his extensive experiments, to which reference has already been made, in terms of the theory of anion respiration. He regarded the outer boundary of the epidermis as the primary barrier to the free entry of ions.

At present the majority of investigators favour the view that the accumulation of ions within the vacuoles of cells is mediated by a carrier mechanism. This concept appears to have been first suggested by Wohl & James (37) and was subsequently discussed in considerable detail by Overstreet & Jacobson (38). The numerous types of carrier mechanisms that have been proposed were recently reviewed by Laties (22).

Relatively little consideration, however, has been given to the possible importance of carrier mechanisms in the transfer of ions across the roots of intact plants. However, Russell *et al.* (27) observed that whereas metabolic inhibitors reduced the transfer of phosphate to the shoots of plants when the roots were bathed in relatively concentrated solutions (e.g., 10 p.p.m. phosphate), the reverse could occur when the concentration in the outer medium was very low (e.g., 0.01 p.p.m. phosphate). They attributed the enhanced upward movement to the fact that the inhibitors reduced the extent to which phosphate was retained in roots by metabolic processes, so that larger quantities are thereby available for upward movement. This implies that the quantity of preformed carrier was insignificant in relation to the quantities of phosphate which would normally be transported when plants were supplied with more concentrated solutions and for this reason inhibitors did not then enhance upward movement. While this interpretation is speculative it does not seem possible to attribute the observations of Russell *et al.* (27) to the passive movement of phosphate in water across the root [*vide* Laties (22)]. When roots are bathed in such dilute solutions the retention of ions by exchange as opposed to their diffusion in water can be expected to be maximal and it is only under these conditions that inhibitors increase the transfer of ions to the shoot. Moreover, the complex biochemical reactions into which recently absorbed phosphate enters rapidly, make the possibility of free diffusion appear remote [Loughman & Russell (39)].

The evidence so far considered is discordant in a number of respects and indeed some of the interpretations referred to are now little regarded. However, the conclusion is strongly suggested that the transfer of ions across the symplast of the intact root into the vascular stele depends on the expenditure of respiratory energy and that different species of ions move at their own independent rates. None the less, the existence of some close

relationship between transpiration and the overall process whereby ions reach plant shoots has been indicated by investigations in which the accumulation of nutrients in shoots was reduced by the curtailment of transpiration.

Such relationships were largely elucidated by Broyer & Hoagland (3), who in their first series of experiments, allowed plants of low salt content and high sugar status to absorb potassium and bromide for periods of 12 hr. under conditions of high humidity in darkness or low humidity in light. The root temperature was kept constant at 25°C. Under conditions of low humidity in light the rate of transpiration was over three times greater than that observed with high humidity in darkness, but the rate at which the potassium and bromide ions were absorbed was only little affected (Table I).

TABLE I

EFFECTS OF TRANSPIRATION ON THE ABSORPTION OF THE POTASSIUM AND BROMIDE IONS BY INTACT PLANTS UNDER DIFFERENT CONDITIONS

[Data of Broyer & Hoagland (3)]

Experimental Conditions	Absorption of Water	Absorption of Salt	
		Potassium	Bromide
	(*ml./gm. fresh weight of shoot*)	(*mg.* $\times 10^2$*/gm. total fresh weight*)	
Low Salt, Low Humidity, Light	9.60	10.85	9.52
Low Salt, High Humidity, Light	3.60	10.40	9.65
Low Salt, High Humidity, Dark	2.52	8.75	9.13
High Salt, Low Humidity, Light	8.10	5.20	6.07
High Salt, High Humidity, Light	2.58	3.24	4.24
High Salt, High Humidity, Dark	1.49	1.39	2.15

When, however, similar experiments were carried out using plants of high salt content and low sugar status, contrasting results were obtained and the rate of ion uptake varied in much the same manner as the rate of transpiration (Table I). Broyer and Hoagland concluded that the transfer of ions across the plant roots was an active process, dependent on respiration, but that the subsequent upward movement of ions to the shoots occurred passively in the water stream. In the plants of high salt status the latter process was considered rate limiting and consequently, variation in the rate of transpiration affected the rate of the overall process. These workers also noted that when roots were injured by placing them in a hypertonic single salt solution they behaved like wicks and salt uptake to the shoot varied strictly in proportion to water uptake. The contrasting behaviour of uninjured plants suggested that this treatment had destroyed the barrier across which ions are normally transferred by a metabolic process. Apart from providing strong evidence that ions are not passively transferred across

the root, the work of Broyer and Hoagland showed clearly that the relationship between salt uptake and transpiration may vary greatly depending on the nutrient status of the plant. Therefore, little progress is likely to be made in the study of their interaction unless this factor is borne in mind.

Subsequently, Brouwer (5) employed a micro-potometer to obtain information on the absorption of water and chloride ions by 7-day-old *Vicia faba* seedlings. He found no positive correlation between the absorption of water and of salt. The independence of the two processes was illustrated by using respiratory inhibitors and by increasing the osmotic level in the external medium; the application of 10^{-5} *M* dinitrophenol inhibited the uptake of chloride without affecting that of water while the absorption of water but not of chloride was reduced by the latter treatment. Van den Honert *et al.* (13) investigated the relationship between transpiration and the absorption of potassium, nitrate, and ammonium ions in maize plants. They concluded that transpiration did not exert a direct effect on ion uptake and they cited results of experiments with tropical crops which supported this view.

Russell & Shorrocks (11, 12) carried out experiments in many respects similar to those of Broyer and Hoagland. The use of tracer methods, however, enabled them to employ considerably wider ranges of concentrations in the external solution (0.3 to 85 p.p.m. rubidium and 0.1 to 93 p.p.m. phosphate); moreover it was possible unequivocally to distinguish nutrients absorbed during the short experimental periods from those which had entered previously. The rate of transpiration was varied by exposing plants to light or darkness and evidence was presented to show that the effects of darkness could be validly attributed to reduction in the rate of transpiration.

When plants of low initial nutrient status were maintained in dilute solutions the transfer of both rubidium and phosphate to shoots was little affected by transpiration; however, the transfer of ions to shoots varied closely with transpiration in plants of high salt status supplied with relatively concentrated solutions. The effects observed by Broyer & Hoagland were therefore confirmed. Russell & Shorrocks attached particular importance to comparing the concentration of ions in the vascular sap with that in the external medium. For this purpose they calculated "transpiration stream concentration factors" which they defined as the ratio of the concentration in the transpiration stream to that in the external solution. They calculated the mean concentration in the transpiration stream from the amount of water transpired and the quantity of labelled ion transferred to the shoot during experiments which usually lasted 24 hr. or less. This procedure involved the assumption that phosphate and rubidium moved from roots to shoots as inorganic ions in solution; they cited evidence in support of this view [Stout & Hoagland (40); Tolbert & Wiebe (41); Loughman & Russell (39)].

A transpiration stream concentration factor of one would indicate that

the ion under investigation moved from the external medium to the shoot at the same rate as water while values below one would indicate that the ion moved more slowly. Both these effects could occur as the result of free diffusion. However, values in excess of one would show that the ions had moved against a concentration gradient and it was argued that the occurrence of such values would be conclusive evidence that the ions had been

TABLE II

SUMMARY OF TRANSPIRATION STREAM CONCENTRATION FACTORS OBSERVED IN EXPERIMENTS WITH INTACT PLANTS

Ion	Concentration in External Medium	Plant	Transpiration Stream Concentration Factor		Source
			High Transpiration	Low Transpiration	
	(*p.p.m.*)				
Phosphate	0.1	Barley	12.	156.	Russell & Shorrocks (11, 12)
Phosphate	0.1	Barley	24.	108.	Russell & Shorrocks (11, 12)
Phosphate	0.1	Sunflower	34.	145.	Russell & Shorrocks (11, 12)
Phosphate	0.1	Sunflower	11.	50.	Russell & Shorrocks (11, 12)
Phosphate	31.	Barley	1.1	3.3	Russell & Shorrocks (11, 12)
Phosphate	31.	Barley	0.6	2.3	Russell & Shorrocks (11, 12)
Phosphate	31.	Sunflower	0.6	1.4	Russell & Shorrocks (11, 12)
Phosphate	31.	Sunflower	0.5	0.9	Russell & Shorrocks (11, 12)
Rubidium	0.31	Barley	36.	73.	Russell & Shorrocks (11, 12)
Rubidium	0.28	Barley	20.	32.	Russell & Shorrocks (11, 12)
Rubidium	1.34	Sunflower	3.7	2.7	Russell & Shorrocks (11, 12)
Rubidium	85.	Barley	2.3	8.4	Russell & Shorrocks (11, 12)
Rubidium	85.	Barley	3.6	2.2	Russell & Shorrocks (11, 12)
Rubidium	85.	Barley	1.8	2.6	Russell & Shorrocks (11, 12)
Rubidium	85.	Barley	0.8	0.7	Russell & Shorrocks (11, 12)
Rubidium	85.	Sunflower	1.1	0.7	Russell & Shorrocks (11, 12)
Sulphate	4.5	Wheat	11.		Kylin & Hylmö (10)
Sulphate	45.	Wheat	1.8		Kylin & Hylmö (10)
Sulphate	450.	Wheat	0.41		Kylin & Hylmö (10)
Sulphate	4.5	Wheat	10.		Kylin & Hylmö (10)
Sulphate	45.	Wheat	1.9		Kylin & Hylmö (10)
Sulphate	450.	Wheat	0.62		Kylin & Hylmö (10)
Sulphate	4.5	Wheat	7.9		Kylin & Hylmö (10)
Sulphate	45.	Wheat	1.2		Kylin & Hylmö (10)
Sulphate	450.	Wheat	0.60		Kylin & Hylmö (10)

transferred in consequence of the expenditure of metabolic energy. They found (Table II) that the transpiration stream concentration factor varied greatly depending both on the external concentration of the ion in question and on the nutrient status of the plant. The highest values, in some cases in excess of 100, were obtained when plants were absorbing from dilute solutions in the dark; the lowest occurred in those absorbing from solutions of high concentration in the light. They pointed out that Kylin & Hylmö (10), who favoured the view that ions are passively transferred

across the root, none the less, had published data which showed that transpiration stream concentration factors of approximately 10 could occur when wheat seedlings were allowed to absorb sulphate from dilute solutions in darkness (Table II). Other evidence that the concentration of ions in the aqueous phases in the vascular sap can exceed that in the outer medium has previously been provided by examination of the exudate from decapitated roots [Lundegårdh (24); Broyer (28)].

Russell & Shorrocks concluded that higher concentrations of ions in the transpiration stream than in the outer medium, under conditions of low water movement, showed that ions are not able to pass by mass flow across the symplast at a rate comparable to that at which they are transferred by active transport. They did not exclude the possibility that slight passive movements could occur, but they considered it unimportant from the viewpoint of the nutrition of intact plants.

The Postulate That Ions Are Passively Transferred Across Plant Roots

Between the time when Steward (18) and Hoagland & Broyer (19) demonstrated the metabolic nature of ion accumulation and 1953 when Hylmö (7) suggested that mass flow in the transpiration stream was the major mechanism whereby ions reach the shoots of intact plants, the assumption that ions are transferred across the roots of intact plants by a process dependent on metabolism appears to have been generally accepted. Both the design and the results of Hylmö's experiments resemble some of those which earlier led Broyer & Hoagland (3) to the contrary conclusion. Hylmö chose 3-week-old pea seedlings as his experimental material and found that the absorption of calcium and chloride ions fluctuated with the rate of water loss when plants were grown under conditions favouring high and low transpiration. Although Broyer & Hoagland had shown that the nature of the relationship between transpiration and the accumulation of salts may vary with the nutrient status of plants, Hylmö did not consider this factor. No information is given on the ionic status of the plants he used. However, because of the considerable reserves of nutrients present in the seeds of peas and the culture methods employed, it may be assumed that his plants were of relatively high salt status. Broyer & Hoagland had shown that this condition can cause the transfer of ions to plant shoots to vary with the rate of transpiration.

In discussing his results, Hylmö recognised three mechanisms of absorption namely: (*a*) rapid uptake of ions into Donnan systems where they remained exchangeable, (*b*) metabolic accumulation across the tonoplast of individual cells, and (*c*) the transfer of ions across the root by mass flow in the transpiration stream. The close relationship he observed between transpiration and salt uptake led him to conclude that mass flow provided the major pathway of absorption. In 1955, after Brouwer (5) had published the paper referred to in the previous section, Hylmö (8) attempted to

recalculate his results and claimed that they provided evidence of the passive transfer of ions in the aqueous stream across the root. Brouwer (6) subsequently disputed Hylmö's conclusions and presented additional experimental results to support his view that ions are taken up predominantly by an active mechanism, though the passage of a small component in the water stream was considered possible. Hylmö (9) rejected Brouwer's conclusions.

It is unnecessary to discuss this controversy in detail, but the publicity Hylmö's views have received [Epstein (15, 16); Kramer (17)] makes relevant a careful examination of the manner in which he reached his conclusions. There can be no disagreement with his statement "the author's experiments constitute further evidence of the view that under certain well definable conditions the transpiration influences the uptake of ions by the plant" [Hylmö (7), p. 380]. Such a statement might equally well have been made by Broyer & Hoagland (3). Hylmö, however, went on to state "the linear relationship between water and ion transport means that the true concentration of the transpiration stream is the same for all rates of water transport." It is true that he consistently found that the quantity of ions reaching plant shoots increased with the rate of water loss, but his statement is open to question on two grounds. First, in those experiments for which he gave results for absorption at more than two rates of transpiration, the relationship between absorption and transpiration frequently showed considerable divergence from linearity; in the absence of statistical analysis the significance of these divergencies cannot be assessed. Second, on the graphic representations of his results, the lines showing the relationship between absorption and transpiration seldom passed through the origin and suggest that appreciable absorption would have occurred when transpiration was zero. Thus, the concentration in the transpiration stream did not remain constant but decreased with increasing transpiration. On the basis of the linear relationships he proposed it was possible to assign the nutrients which reach shoots, under any given conditions of transpiration, into two components, defined as: Component A—that which would be absorbed by a metabolic mechanism in the absence of transpiration, and Component B—the extra quantity absorbed as a result of the concurrent transpiration.

Hylmö assumed that this kind of calculation permitted him to distinguish quantitatively between two separate mechanisms whereby ions are transferred across the roots of the plant; namely, the metabolic activity responsible for Component A, and the passive movement in the transpiration stream responsible for Component B. This type of argument is the basis of the detailed analyses he has published both of his own considerable data and of that of Brouwer (5). Confidence in his treatment of the latter is reduced by the extrapolations and assumptions which he made.

There can be no denial that if Hylmö's data is examined in isolation from the findings of other workers, his interpretation has the apparent attraction

of simplicity. However, an alternative interpretation is equally consistent with his results, namely that both ionic components are transferred across the root by the same mechanism, but that under the conditions of his experiments, the rate at which ions diffused in water during one stage of the overall process was rate-limiting so that increased transpiration enhanced the overall rate of transfer. Faced with alternative interpretations it is prudent to examine them in the light of all available data relating to the subject. Although Hylmö's conclusions are at variance with the evidence already reviewed—that ions are transferred across plant roots by a process dependent on metabolism—he offers no detailed analyses of the causes of these divergent views. Some of his own data do conflict with his theory; it has already been noted that one of his results provided evidence that the concentration of ions in the transpiration stream may considerably exceed that in the outer medium [Kylin & Hylmö (10)].

The reason why Hylmö and those who have quoted his views [Epstein (15, 16); Kramer (17)] have apparently not considered alternative interpretations of his results nor felt it necessary to obtain experimental evidence by other methods is not far to seek. The concept of "apparent free space" had been enunciated shortly before he undertook his investigations and the passive transfer of ions across roots appeared readily compatible with this hypothesis. It is therefore necessary to examine the concept of apparent free space in relation to the transfer of nutrients across the roots of intact plants.

The Significance of Apparent Free Space in Relation to the Transfer of Ions Across Plant Roots

During the past decade no new concept has proved more stimulating in the study of ion absorption than that of "apparent free space" (AFS) which owes its origin to Briggs. Recent detailed discussion [Briggs & Robertson (42); Briggs (43)] make it unnecessary to review this theory except as it is relevant to assessing the situation in intact plant roots. It appears beyond doubt that a considerable volume of cortical tissues, whether sliced storage tissue or root segments, is readily accessible for the entry of ions by Donnan exchange or diffusion. The extent and the location of the free space however, is less certain. In the different tissues it has been estimated to account for from 8 to over 30 per cent of the total tissue volume [Hylmö (7); Levitt (44); Butler (45); Epstein (46)]. Some investigators have suggested that it includes cytoplasm external to the tonoplast [Hope & Stevens (47); Hope (48); Lundegårdh (21, 49); Briggs *et al.* (50)] while others consider that it is mainly or entirely located in the intercellular spaces and in the cell walls [Levitt (44); Keller & Deuel (51); Jansen *et al.* (52); Hope & Dainty (53)]. Despite these uncertainties it would seem probable that free space exists throughout the cortical tissue of young roots so that when the flux of ions is sufficient to prevent their retention by exchange reactions on static sites, and when they are not actively accumulated in the vacuoles of cells,

they can pass freely by mass flow in water to the endodermis at the inner boundary of the cortex.

In relation to the present subject, the question of paramount importance is whether ions can likewise traverse the endodermis in "free space." No direct evidence is available on this question; in higher plants apparent free space has been demonstrated in cortical tissues only. One is therefore forced to speculation based on the histological characteristics of the endodermis. Its unique structure has been described by many investigators [Bond (54); Warden (55); Guttenberg (56)]. It is a single layer of cells, the long axes of which lie parallel to that of the stele, and there are no intercellular spaces. The Casparian strip, a band of thickening, occurs in the cell walls and is developed to different degrees in different groups of plants. Whereas in the gymnosperms and dicotyledons it is normally developed on the end and radial walls, in monocotyledons it is more pronounced on the radial and tangential walls. The thickening consists of a waxy substance, similar to cutin and suberin, which takes histological stains for lignin to some degree and is therefore commonly referred to as lignosuberin; it is considered impervious to water. The Casparian strip is traversed by pits through which the cytoplasm is so well connected that on plasmolysis it remains attached to the tangential walls giving the appearance of a continuous ribbon of cytoplasm ringing the endodermis [Bryant (57)]. Unthickened or "passage" cells occur in the endodermis; in roots they are situated opposite the protoxylem.

The question to be considered here is whether or not these features of the endodermis so distinguish it from cortical tissues that it is unlikely to share with them the characteristics of "free space." If the hypothesis is accepted that "free space" is located outside the cytoplasm in cortical tissues then it would seem reasonable to assume that there is no "free space" in endodermal cells, except possibly in the passage cells, since intercellular spaces are lacking and the Casparian strip is impervious to water. If, however, "free space" is situated partially or entirely in the cytoplasm the situation is less certain.

There is a general belief that the properties of the cytoplasm of all cells are similar. None the less, even within a single cortical cell it is well accepted, by those who believe in the existence of the cytoplasmic free space, that the tonoplast is a unique zone of the cytoplasm being a barrier to free ionic movement. The appearance of plasmolized endodermal cells differs so strikingly from that of normal cortical cells, that it seems not unreasonable to suspect that they may contrast as greatly in their behaviour to the passage of ions. There is no basis for advancing one conclusion or the other. Even, if the rather attractive, though teleological, argument that the special form of the endodermis denotes special functions is rejected, it can at least be stated that there is no direct evidence for the existence of free space in the endodermis similar to that in the cortex. Certainly no assertion to the contrary is justified. The fairest conclusion appears to be that the study of free space, as it has so far progressed, has little relevance to the discussion of the behaviour of the endodermis and hence of the mechanism whereby

ions are transferred to the vascular stele of an intact root, except in so far as it indicates that there may be no high resistance barrier in the cortex.

Conclusions

The conclusions appearing most reasonable in the light of present evidence may now be summarised.

(*a*) Ions can enter into the free space of the cortical tissues of roots, though other mechanisms whereby ions may be transferred across the cortex cannot be excluded.

(*b*) At some sites external to the vascular tissue there is a barrier to the free movement of ions which, under some circumstances, is capable of maintaining considerably higher concentrations of ions within the vascular stele than in the ambient medium.

(*c*) The transfer of ions across this barrier depends on the expenditure of metabolic energy.

(*d*) Some step in the overall process whereby ions are transferred from the outer medium to the shoot can, under certain circumstances, be accelerated by increasing the rate of transpiration.

It is relevant to consider whether the process of diffusion in water which can be stimulated by transpiration occurs outside the high resistance barrier or within the vascular tissue itself. If it were located externally it would be expected that enhanced water movement would have the greatest effect on the overall process when absorption would otherwise be limited by the low quantity of salt reaching the site of active transport; that is, when the concentration of ions available for absorption is low. The minimum effect would be expected when the concentration of ions is so high that any further increase in concentration would cause little, if any, increment in metabolic movement. This is not borne out by the experimental data; increased transpiration has its greatest effect on the upward movement of ions when the concentration of ions is high both within and external to the tissue (3, 11, 12). This situation is compatible with the view that transpiration can stimulate salt uptake by hastening the upward transfer to shoots of ions already actively transported across a high resistance boundary and which would otherwise reduce the rate of that transfer by virtue of an excessive difference in concentration between the two sides of the barrier. The analogous manner in which concentration differences may limit accumulation in tissue slices has been proposed by Sutcliffe (58).

From the foregoing it is apparent that any discussion of the mechanism whereby ions are transferred across the roots of intact plants must be highly speculative. The reason for this lies largely in the difficulty of carrying out detailed physiological experiments on complex tissues. However, it is perhaps legitimate to speculate whether more progress might not have been made if the easier experimental manipulation of the simpler tissue systems and the greater ease with which the results can often be interpreted, had not caused many workers to reject the intact plant as material for precise

experimentation. The disadvantage of the intact plant from this viewpoint is obvious; individual processes cannot readily be studied in isolation. The phenomena observed usually result from many sequential steps, sometimes occurring in different tissues, and as Blackman (59) realised many years ago, the rate of the overall process may largely be controlled by any single step if it happens to be rate limiting. Under these circumstances observations made when a single variable is alone considered, may readily mislead; the effects of all other relevant variables must be examined if the operative mechanism is to be adequately interpreted. The elucidation of the relationship between transpiration and salt uptake which occurred when Broyer and Hoagland varied both transpiration and the nutrient status of plants is illustrative of this principle.

The conclusion may also be drawn that results obtained in experiments with simple tissue systems do not, by themselves, provide an adequate basis for interpreting relationships in the more complex system of the intact plant.

LITERATURE CITED

1. Wright, K. E., *Plant Physiol.*, **14,** 171 (1939)
2. Phillis, E., and Mason, T. G., *Ann. Botany* (*London*), **4,** 645 (1940)
3. Broyer, T. C., and Hoagland, D. R., *Am. J. Botany,* **30,** 261 (1943)
4. Michael, G., and Wilberg, E., *Z. Pflanzenernähr. Düng. Bodenk.,* **52,** 242 (1951)
5. Brouwer, R., *Acta Botanica Neerl.,* **3,** 264 (1954)
6. Brouwer, R., *Acta Botanica Neerl.,* **5,** 287 (1956)
7. Hylmö, B., *Physiol. Plantarum,* **6,** 333 (1953)
8. Hylmö B., *Physiol. Plantarum,* **8,** 433 (1955)
9. Hylmö, B., *Physiol. Plantarum,* **11,** 382 (1958)
10. Kylin, A., and Hylmö, B., *Physiol. Plantarum,* **10,** 467 (1957)
11. Russell, R. S., and Shorrocks, V. M., *Radioisotopes in Scientific Research,* **4,** 286; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 1958)
12. Russell, R. S., and Shorrocks, V. M., *J. Exptl. Botany,* **10,** 301 (1959)
13. Honert, T. H. van den, Hooymans, J. J. M., and Volkers, W. S., *Acta Botanica Neerl.,* **4,** 139 (1955)
14. Arisz, W. H., *Protoplasma,* **46,** 5 (1956)
15. Epstein, E., *Ann. Rev. Plant Physiol.,* **7,** 1 (1956)
16. Epstein, E., *Science,* **124,** 937 (1956)
17. Kramer, P. J., *Science,* **125,** 633 (1957)
18. Steward, F. C., *Protoplasma,* **18,** 208 (1933)
19. Hoagland, D. R., and Broyer, T. C., *Plant Physiol.,* **11,** 471 (1936)
20. Robertson, R. N., *Ann. Rev. Plant Physiol.,* **2,** 1 (1951)
21. Lundergårdh, H., *Ann. Rev. Plant Physiol.,* **6,** 1 (1955)
22. Laties, G. G., *Ann. Rev. Plant Physiol.,* **10,** 87 (1959)
23. Shapiro, R. E., Taylor, G. S., and Volk, G. W., *Soil Sci. Soc. Am. Proc.,* **20,** 193 (1956)
24. Lundegårdh, H., *Arkiv För Botanik,* **31A,** 1 (1943)
25. Robertson, R. N., and Turner, J. S., *Australian J. Exptl. Biol. and Med Sci.,* **23,** 63 (1945)
26. Robertson, R. N., Wilkins, M. J., and Weeks, D. C., *Australian J. Sci. Research,* **B4,** 248 (1951)
27. Russell, R. S., Martin, R. P., and Bishop, O. N., *J. Exptl. Botany,* **4,** 136 (1953)
28. Broyer, T. C., *Plant Physiol.,* **25,** 367 (1950)
29. Lundegårdh, H., *Arkiv. Botan.* **32A,** 1 (1945)
30. Hoagland, D. R., *Soil Sci.,* **16,** 225 (1923)
31. Collander, R., *Plant Physiol.,* **16,** 691 (1941)
32. Jacobson, L., and Overstreet, R., *Soil Sci.,* **65,** 129 (1948)
33. Rediske, J. H., Cline, J. F., and Selders, A. A., *Hanford Works Report,* **HW36734** (1955)
34. Priestley, J. H., *New Phytologist,* **19,** 189 (1920)
35. Crafts, A. S., and Broyer, T. C., *Am. J. Botany,* **25,** 529 (1938)
36. Lundegårdh, H., *Physiol. Plantarum,* **3,** 103 (1950)
37. Wohl, K., and James, W. O., *New Phytologist,* **41,** 230 (1942)
38. Overstreet, R., and Jacobson, L., *Ann. Rev. Plant Physiol.,* **3,** 189 (1952)
39. Loughman, B. C., and Russell, R. S., *J. Exptl. Botany,* **8,** 280 (1957)
40. Stout, P. R., and Hoagland, D. R., *Am. J. Botany,* **26,** 320 (1939)
41. Tolbert, N. E., and Wiebe, H., *Plant Physiol.* **30,** 499 (1955)

42. Briggs, G. E., and Robertson, R. N., *Ann. Rev. Plant Physiol.*, **8**, 11 (1957)
43. Briggs, G. E., *New Phytologist*, **56**, 305 (1957)
44. Levitt, J., *Physiol. Plantarum*, **10**, 882 (1957)
45. Butler, G. W., *Physiol. Plantarum*, **6**, 617 (1953)
46. Epstein, E., *Plant Physiol.*, **30**, 529 (1955)
47. Hope, A. B., and Stevens, P. G., *Australian J. Sci. Research, B*, **5**, 335 (1952)
48. Hope, A. B., *Australian J. Biol. Sci.*, **6**, 396 (1953)
49. Lundegårdh, H., *Physiol. Plantarum*, **11**, 585 (1958)
50. Briggs, G. E., Hope, A. B., and Pitman, M. G., *J. Exptl. Botany*, **9**, 128 (1958)
51. Keller, V. P., and Deuel, H., *Z. Pflanzenernähr. Düng. Bodenk.*, **79**, 119 (1957)
52. Jansen, E. F., Jang, R., Albersheim, P., and Bonner, J., *Plant Physiol.* (In press)
53. Hope, A. B., and Dainty, J., *Australian J. Biol. Sci.*, **12**, 395 (1959)
54. Bond, G., *Trans. Roy. Soc. Edinburgh*, **56**, 695 (1931)
55. Warden, W. M., *New Phytologist*, **36**, 361 (1935)
56. Guttenberg, H. von, in *Handbuch der Pflanzenanatomie*, **5**, 24 (Linsbauer, K., Ed., Gebrüder Borntraeger, Berlin, Germany, 1943)
57. Bryant, A. E., *New Phytologist*, **33**, 231 (1934)
58. Sutcliffe, J. F., *J. Exptl. Botany*, **3**, 59 (1952)
59. Blackman, F. F., *Ann. Botany* (*London*), **19**, 281 (1905)

TRANSPORT IN THE XYLEM[1,2]

By E. G. Bollard

Fruit Research Division, Department of Scientific and Industrial Research, Auckland, New Zealand

No longer is it possible to picture translocation in plants as consisting simply of an upward movement of absorbed mineral elements in the xylem and a downward movement of elaborated nutrients in the phloem. It is now known that organic compounds move in the xylem and at least some inorganic ions apparently move in the phloem. Also, it is known that plants can absorb many complex organic compounds through the roots and that many of these compounds are distributed through the plant in the xylem. In the past, translocation has usually been discussed from the point of view of movement within plants of either inorganic ions or organic molecules (6, 121, 174), but in this volume the subject is approached from the separate points of view of the two conducting tissues principally involved.

This review will deal with the movement of absorbed nutrients into and through the xylem and their distribution from the termini of this system. Entry of substances into the xylem of the plant root has proved difficult to study experimentally and as yet there is no clear idea of the mechanisms involved. With many plant species it is now possible to extract sap from the xylem elements and examination of this liquid has revealed the presence of many organic compounds. By using radioisotopes it has been possible to study the redistribution of some elements within plants after their delivery into the transpiration stream. There is now evidence that some compounds applied externally to leaves can enter and move in the phloem and that subsequent movement to the xylem can also occur.

Problems of water movement into the xylem, of ascent of sap (37, 82), and of pathological conditions affecting transport in the xylem (70) have been recently reviewed and will not be discussed here.

ENTRY OF NUTRIENTS INTO THE XYLEM

Before entering the xylem elements, salts absorbed from the exterior have to cross the epidermis, cortex, endodermis, and vascular parenchyma of the root. No mechanism has yet been established for the passage of nutrients across these outer tissues of the root, nor is their precise route known. No direct method of studying the entry of salts into the xylem has been devised, though definition of the particular region of the root where entry into the xylem occurs has been possible. Most recent ideas on the mechanism of movement of absorbed ions through the root cortex derive

[1] The survey of literature pertaining to this review was concluded in August 1959.

[2] The following abbreviations will be used: DTPA (diethylenetriamine pentaacetic acid); EDTA (ethylenediamine tetraacetic acid); 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).

from studies on ion uptake by disks of parenchyma tissue or entire root systems.

Region of salt entry in xylem.—When portions of roots are exposed to indicator ions such as radioisotopes, the accumulation of such ions can be detected over much of the length of the root where the epidermis remains unsuberized. If roots treated in this way are returned to nonradioactive liquids, radioactivity disappears from some regions and it has been assumed that these are the zones in which nutrients move from cortex to xylem.

Wiebe & Kramer (198) exposed a sequence of 3 mm. lengths of intact root of barley seedlings to a number of labelled ions. Translocation occurred from a region 10 to 60 mm. behind the root tip, with greatest activity occurring about 30 mm. from the tip. Root tips accumulated ions freely but little translocation from the terminal 5 mm. region of the roots was detected.

Using labelled phosphate, Canning & Kramer (51) observed similar translocation from portions of the roots of corn seedlings ranging up to 80 mm. from the root tips. In the same series of experiments cotton and pea seedlings showed significant translocation from the root tip area as well as from areas up to 55 mm. behind the root tips. These results suggest that salts move into the xylem over a region several centimetres long behind the root tip. In much of this region the vascular elements of the xylem are mature and, as Kramer & Wiebe point out, it is the region where maximum water absorption occurs.

Mechanism of entry of nutrients.—It has been established for some time (89) that the uptake of ions by roots depends on their active respiration. Also, (47) the concentration of salts in exuded sap from excised roots can be many times that in the solution in which the roots grew.

Several theories have been proposed to explain the mechanism of movement of salts into the xylem against such a concentration gradient and how the necessary energy can be derived from respiratory reactions. These theories have been discussed in detail recently (173) and need only be mentioned here. The theories of Crafts & Broyer (56) and Lundegårdh (115) both suggest that incoming ions are accumulated in the epidermal cells of the root and that subsequent movement into the stele is along a gradient. Ions are then supposed to be released into the xylem vessels because of lower oxygen tensions in the centre of the root, but, as Sutcliffe (173) points out, the existence of such oxygen deficits is doubtful. On the other hand, Arisz (5) suggests that passive movement of salts may occur across the root cortex and that the only energy-requiring step necessary is to transport the ions from the protoplasmic layer of the adjacent living cells into the cavities of the vascular elements of the xylem.

The concept of free space in a cell or tissue, i.e., the part into which the solute and solvent from the external solution can penetrate readily (41) has recently dominated much of the discussion on ion movement into cells and tissues. Kramer (103) has discussed some of the implications of this concept on current ideas of ion movement through the outer root tissues and

into the vascular system. He pointed out that this free space, which probably includes both cell walls and much of the cytoplasm, provides a pathway by which ions could move from the soil solution into the root without passing through a vacuole or being accumulated by any cell. On this basis the cavities of the xylem vessels would be part of the free space and continuous with the cortical free space. Movement into and through the free space, once a steady state had been attained, would be nonselective—all the compounds of the soil solution might be expected to move into the free space and into the transpiration stream.

According to this view, solutes passing through the cortical free space could be removed from the general stream of substances moving into the xylem by accumulation into cell vacuoles. Whereas movement into the free space would be nonselective, accumulation would be selective and Kramer considered it possible that the known differences in elemental composition between different plant species could be accounted for by differential accumulation of the elements concerned. However, if the same soil solution is taken up by different plants, then for species to differ in final composition ions must be lost differentially from different species. It is well known (201) that substances can be leached from the foliage of plants but it is not established that either this leaching or some other loss occur in quantities sufficient to explain the marked differences that occur, for example, between species of *Astragalus* which may or may not accumulate selenium (128).

As he considered it unlikely that plant cells accumulated large molecules, Kramer (103) thought that movement of solutes in free space probably explained the known movement of such large molecules as iron chelates and antibiotics in plants. The two examples given by Kramer, griseofulvin and iron chelates, have molecular weights of 356 and 445 respectively. It is now known, however, that sucrose (mol. wt. 342) (143) and streptomycin (mol. wt. 581) (149) can be accumulated by plant cells. Although molecular weight is not the only criterion for "largeness" of molecules it is clear that some large molecules can be accumulated and there is no need to hypothesize their movement in free space.

Much evidence for passive movement of external nutrients through the root cortex and into the vascular elements of the xylem depends on the increased uptake of solutes which may accompany increased absorption of water. If ions move in free space to the xylem they might be expected to be moved there more rapidly by a more rapid inward movement of water (103). The relation of salt uptake to water uptake is reviewed elsewhere in this volume; we are concerned here with its relevance to the mechanism of salt entry into the xylem. In experiments studying the relation of salt and water uptake, ion absorption of plants has usually been measured under varying conditions of transpiration and in the presence and absence of respiratory inhibitors (42, 43, 92, 93, 94, 102, 161).

Despite much argument over interpretation of experimental results it would appear that there is now agreement that two components are involved in ion uptake by roots. One, the active component, depends on the respira-

tion of the tissues, while the other, the passive component, is directly dependent on movement of water through the root. There is still disagreement, however, as to the relative importance of the two components.

It is also known that the previous history of root systems may affect their capacity for salt absorption. Hoagland & Broyer (89) showed that with roots having a low salt and high sugar content large differences in transpiration rate had little influence on salt absorption or salt movement to the shoot. On the other hand, with high-salt and low-sugar plants, which had a low initial capacity for salt absorption, variations in rate of transpiration had a marked effect on salt movement. To obtain more precise data on these points Russell & Shorrocks (163) measured the effect of varying transpiration rate, concentration of external solution, and salt status of the plant on uptake of both phosphate and rubidium by barley plants. With low-salt plants and dilute external solutions, uptake was independent of transpiration or only slightly affected. With high-salt plants and more concentrated external solutions there was a closer correlation between transpiration and salt uptake. These workers agree with Broyer & Hoagland (47) that the over-all transfer of ions from an external medium to the shoot is not controlled by transpiration. With plants of high salt status, however, some step in this transfer (which may become limiting) can be influenced by the rate of transpiration. This independence of transpiration and salt uptake, at least under some conditions, makes it unlikely that salt moves into the xylem through free space.

Many years ago the suggestion was made (164) that the root endodermis provides a physical barrier to diffusion of solutes. The radial walls of the endodermal cells usually possess suberized bands which, it is considered, should render them impermeable to water and solutes; thus any ions crossing this layer of cells would have to pass through the cytoplasm. However, if the free space of a plant root includes the cell cytoplasm it is difficult to see what barrier to diffusion is offered by this layer of cells (103). Moreover, it is known that the endodermis can be pierced by root initials and that the walls of endodermis cells can be permeated with plasmodesmata.

Despite this apparent inability of the endodermis always to provide a barrier to inward diffusion of solutes, evidence continues to be produced that a part of the intact root is not readily accessible to diffusing substances. Jacobson *et al.* (97) compared the apparent free space of excised barley roots and roots of intact plants as measured by the uptake of phosphate, obtaining appreciably larger values for the excised roots. The volume of the barley root occupied by the stele could account for the increased apparent free space in the excised root, leading to the suggestion that in the intact root this region was not directly accessible to the external solute. In their experiments on the uptake of phosphate and rubidium by barley plants, Russell and Shorrocks (163) were able to measure the mean concentration of each nutrient in the transpiration stream during short duration experiments. With external solutions of low concentration, and low transpiration rates, the concentration of either element in the vascular sap was sometimes greater than one hun-

dred times the concentration of the external solution. They considered that this steep concentration gradient, against which ions can be moved, demonstrated the existence in the root of a barrier, possibly the endodermis, with a high resistance to back flow. Further, they believed the gradient precluded any possibility of the transpiration stream being continuous with the solution in the cortical free space. No satisfactory mechanisms have yet been proposed to explain such a concentration of nutrients in the xylem.

A factor apparently ignored in all considerations of the route and mechanism of movement into the xylem is that all nutrients do not occur in the xylem sap in the inorganic form in which they are absorbed from the exterior. For example, all the nitrogen in the xylem sap of many plants occurs in organic combination. This clearly means that in its passage across the root this element has been exposed to the action of a sequence of enzymes. Our knowledge of cell structure and function is not sufficient to enable us to say whether such changes could be brought about during passage of the element through the free space of a cell or whether accumulation of the element into some cell organelle is necessary for these changes. It appears likely, however, that future theories regarding mechanisms of nutrient uptake by roots cannot ignore the concurrent metabolic changes the absorbed nutrients may undergo.

COMPOSITION OF THE TRANSPIRATION STREAM

Most current ideas on water movement through plants picture columns of water drawn up through the plants as a result of water evaporating from the leaves (83). There is little doubt that under most conditions this moving mass of water, the transpiration stream, does flow from roots to leaves, though under conditions of high atmospheric humidity flow in the reverse direction may occur (38). The various substances transported into the xylem in the root are generally considered to be carried up through the plant in solution in this transpiration stream. With many plants, methods are now available for extracting liquid from xylem elements and, in this section, work on the nature and concentration of nutrients in this xylem sap will be summarized.

Methods of Obtaining Xylem Sap

Exudates from plants.—Two types of exudate from plants can be obtained in quantities which permit some examination of their composition.

Many plants growing under conditions favourable for water uptake and unfavourable for transpiration exude liquid from points along the leaf margins. Drops of this guttation liquid can be collected and analysed but the quantities available are small. As an indication of the contents of the xylem, examination of this liquid has very limited possibilities. There is no clear idea of the exact origin of the substances found in this fluid, since in most species liquid leaving the termini of the xylem elements has to pass through a region of living cells in the leaf before reaching a pore in the epidermis (170).

At certain seasons exudation or bleeding of sap is known to occur from

wounds or holes made in some species of trees. Large quantities of sap can often be obtained by this procedure; up to 6 l. per day from some species (101). Methods have been devised (90) which permit such sap to be collected free from bacterial contamination. The species most commonly studied in this way has been *Acer saccharum,* no doubt because of the ease with which sap is obtained during the bleeding season (24 to 27; 81, 90, 99, 123, 124, 133, 142). Other woody species examined in this way include *Betula* (23, 69, 106, 123, 124, 157), *Vitis* (151, 204), and *Carpinus* (49, 50). More recently, bleeding sap in smaller quantities has been obtained from a wider range of tree species including some gymnosperms (157, 202). Most workers have obtained this sap by collection of liquid exuding from holes or from the stumps of excised branches. In some cases (69, 106) sap has been obtained by collection from the proximal ends of roots after excision from the tree but while still *in situ* in the soil.

It is generally assumed now that sap bleeding from cut stems comes from the cavities of exposed xylem elements, but some doubts have been expressed in the past. On the basis of observations of dye movement into the xylem of cut stems of *Acer* from which bleeding had been taking place, James & Baker (98) considered that the xylem contents had been under tension rather than pressure and that exudation had been coming from living cells in the cambial region. Kramer (101) was unable to repeat their results but was able to show that exudation still occurred after bark was stripped off the stump. With certain herbaceous species he was able to observe exudation from individual xylem elements.

When bleeding sap from woody species can be obtained, the quantities available are usually adequate for most analyses. Nevertheless, as a general physiological method, such analyses are of limited use because relatively few species bleed and even with these species the phenomenon takes place for only a comparatively short period in late winter or early spring.

With actively growing herbaceous species, on the other hand, exudate can be collected from the stumps of plants over much of the growing period. Substantially the same method has been used with corn (4, 112, 113, 140, 141, 153), sunflower (4, 84), cucurbits (55, 104, 182), peas (166), tobacco (68), tomato (7, 181 to 185), wheat (114), and cotton (74). The quantities of sap collected by this method are much smaller than from bleeding trees but nevertheless are sufficient for many purposes. Lauritzen (109) obtained increased quantities of exudate from decapitated bean plants by increasing the air pressure around the roots.

The distinction between plant exudations caused by root pressure and those caused by local stem pressures has been made by Kramer (101). In decapitated herbaceous plants and many woody species the observed exudation is undoubtedly the result of root pressure and the liquid collected can be considered to be a sample of the liquid being supplied by the root system to the upper parts of the plant. With some woody species, however, notably the sugar maple, exudation can readily occur from detached stem pieces (119) and it is now thought that such exudation results from pressures de-

veloped locally in the stem. As the processes which develop such local pressures probably also influence the composition of the sap, such material cannot be relied on to give any indication of the composition of sap leaving the roots. For this reason no discussion of the many published analyses of maple sap is made in this review.

Extraction of sap.—In the past, various methods have been used in an attempt to extract quantities of xylem sap as and when desired. For example, Dixon & Atkins (71, 72) were able to obtain small quantities of liquid from vessels of a number of woody species by centrifuging short lengths of wood. Mason & Maskell (120) obtained sap from cut stems of cotton by displacement with a head of water, and Burstrom (49) obtained sap from a beech tree by suction applied to a wound.

A method of more general application has been described by Bennett *et al.* (10) and has since been simplified and extended (29, 33). By applying a vacuum to the lower end of a detached woody shoot and then successively cutting off small pieces from the upper end of the shoot it is possible to extract up to 25 per cent of the total moisture from the wood.

The sap obtained by this technique was believed to be that present in the cavities of xylem vessels and not seriously contaminated by the contents of living cells. A more detailed investigation has been made with apple (29). Removal of all tissues external to the wood before extraction of sap from shoots made no difference either in the quantity of sap extracted or the composition of the sap with respect to most of the major elements studied. If pieces of wood from such shoots are extracted with alcohol the extract contains relative amounts of nitrogenous compounds different from those present in the sap. Sugars were nearly always absent from the sap, while they were always present in high amounts in the alcoholic extracts.

This vacuum extraction method has been used to obtain sap from woody tissues of both deciduous and evergreen species throughout the year (33). Species with vessels of wide aperture, such as many climbers, are particularly easy to extract. Despite the difference in structure of the xylem vascular element in gymnosperms, sap can be readily extracted from them by this method. Many herbaceous plants with no secondary wood development nonetheless have stems rigid enough to withstand the application of vacuum and this method can be used to obtain xylem sap from them. The same holds for some monocotyledonous species with sufficiently robust stems, e.g., *Zea mays*.

Because it can be applied to most woody species at any time of the year, this sap extraction method is of wide applicability but there is still a considerable range of monocotyledonous and other herbaceous species to which it cannot be applied. Collection of exudate from decapitated plants remains the only known method of extracting the xylem contents of such species.

Composition of Xylem Sap

Bleeding sap.—Many analyses for different constituents have been made of samples of bleeding sap from both herbaceous and woody species. No at-

tempt will be made to review these here as summaries of much of the work have been made (101, 170). Such saps are usually clear solutions with a pH between 5 and 6. They generally contain between 0.1 and 0.4 per cent solids of which up to one-third may be ash. The content of individual elements varies considerably but the following figures for major elements in the bleeding sap of corn can be considered average: N, 275 p.p.m.; P, 30 p.p.m.; K, 115 p.p.m.; and Ca, 35 p.p.m. (112). Sugars are frequently present. Some enzyme activity has been reported in sap from vines (151, 204) and birch (123, 124). Certain organic acids (105, 151, 204) and α-keto acids (182, 183) have also been detected. Recent work (157, 182, 183) using chromatographic methods, has facilitated analysis of the various nitrogenous compounds present in bleeding sap. Alkaloids (68, 132, 135, 186) and coumarins (134, 145, 202) have also been detected.

Marked variations can occur in the inorganic composition of bleeding sap. Experiments wth plants grown in culture solution have shown that the composition of the sap can be affected by changing the composition of the external solution. Thus, by changing the chloride in the external solution from 4 to 154 m.eq. per l., Eaton (74) increased the chloride concenration in bleeding sap from cotton plants by about four times. With annual herbaceous species, changes in sap composition are known to occur at different growth phases (112) but this has received little study. With deciduous species a maximum concentration of most inorganic nutrients appears to occur near the beginning of the bleeding period (50, 69, 106). Attempts have been made (4, 104, 112, 140, 141, 153) to use the composition of bleeding sap as a guide to the nutritional status of agricultural crops. Lack of information on factors influencing the nature and concentration of nutrients in such sap, however, has so far prevented wide application of such methods.

Extracted xylem sap.—Although the use of extracted sap in the study of xylem transport has obvious advantages, surprisingly little is yet known about its composition. After the extraction method was first developed, a few conductivity measurements and iron and copper determinations were made on xylem saps from fruit trees (3, 10, 11, 139) but no further use of the method appears to have been made for 20 years.

A detailed study was subsequently made of changes in the major element content of xylem sap from apple trees over a 12-month period (29, 34). All the elements examined (N, P, K, Mg) could be detected at all times in the sap but marked seasonal changes occurred. From a low level during the dormant phase there was up to a tenfold increase in concentration of most elements during the blossoming period. Highest levels for the elements examined were: N, 160 p.p.m.; P, 30 p.p.m.; K, 175 p.p.m.; and Mg, 20 p.p.m. Such levels were sustained for several weeks after which there was a gradual drop in concentration until by leaf fall the levels of all elements were back to the low value characteristic of dormancy. These measurements were of concentrations in sap at the time of sampling and in themselves gave no measure of the amount of each nutrient passing through the xylem. Maximum concentrations of most elements had occurred before the apple trees

were in full leaf, but probably greater amounts of nutrients passed through the xylem later when the sap concentration had decreased but transpiration had increased. Detailed studies have not been made of other species, but from occasional analyses for some elements it would appear that similar seasonal changes occur in many deciduous species. There is no information available concerning the variation in composition of sap in evergreen species.

There is little information on the organic constituents of extracted xylem sap other than the nitrogenous constituents. Sugars are briefly present in apple sap in low concentration about blossoming time (29). No assays of the sap for enzyme activities have been made.

XYLEM TRANSPORT OF MINERAL ELEMENTS

Since the combined ringing and isotope experiments of Stout & Hoagland (172) there has been general agreement with the earlier view that nutrients are carried up the plant from the root system in the xylem. Since then, many ringing experiments (e.g., 36, 191) have been carried out with little interference to the upward movement of nutrients, whereas xylem removal (188, 191) completely prevents upward movement. Occasionally there is a suggestion (188) that ringing can slightly reduce the upward movement of nutrients, but it seems unlikely that this is simply due to removal of the tissue through which the extra movement would take place.

It is generally assumed that all mineral elements move in the xylem, though the ringing experiments referred to used only phosphorus, potassium (172), and nitrogen (36). Analyses of extracted sap have also shown the presence of most other essential elements (3, 11, 29, 34, 139).

The following sections summarize what is known of the forms in which nitrogen, phosphorus, sulphur, and iron are translocated in the xylem. There appears to be no information for the other essential elements on the particular chemical forms in which they occur in the xylem.

Translocation of Nitrogen

Transport of nitrate.—Although plants can use many compounds of nitrogen as a sole source of this element under experimental conditions (35), general agreement exists that plants growing in soil usually take up most of their nitrogen in the form of nitrate. Certainly nitrate can be found in the tissues of some plants in appreciable amounts, especially those growing under low light conditions (9), and it has been generally assumed that nitrate was the form in which nitrogen moved through plants. However, it has been known for a long time (175) that nitrate could only be detected in apple in the smallest roots. In this species reduction of nitrate was assumed to occur in these roots and it has since been shown that in this species nitrogen is translocated through the xylem largely as amino acids.

Fractionation of the nitrogen in the xylem sap extracted from shoots of the apple tree and other rosaceous fruit species showed that, apart from a small proportion of ammonia nitrogen, it could all be accounted for by the sum of amide nitrogen and amino nitrogen (28, 31, 32). Examination of a

wider range of plants indicated that the occurrence of more than a trace of nitrate in the foliage is the exception rather than the rule (30, 33). Nitrate was found to occur in the xylem sap from some species only, and then generally only as a minor constituent of the nitrogen fraction. A variety of amino acids and other soluble nitrogeneous compounds was found in all saps. It is clear now that the idea of nitrate being the main form in which nitrogen is transported through the plant will have to be abandoned. Even in the few species where appreciable nitrate occurred in the xylem sap, considerable amounts of organic nitrogenous compounds were also found. Plants with high concentrations of nitrate in the foliage are usually herbaceous species in which direct examination of xylem saps cannot be made, but even here it is still possible that translocation of organic nitrogen may normally occur and that this is supplemented by greater or lesser amounts of nitrate.

Transport of organic nitrogenous compounds.—The nitrogen in the xylem sap of apple (28, 31) and other rosaceous fruit species (32) consists of a range of amino acids and amides with aspartic acid and asparagine predominating. Despite considerable variations in the level of nitrogen present in the sap throughout the growing season, these two compounds accounted for 70 to 95 per cent of the total. The remainder was usually made up of small amounts of alanine, methionine, valine, leucine, serine, threonine, and γ-aminobutyric acid.

Examination of the nitrogenous constituents in xylem sap obtained from a range of plants showed amino acids always present. In most, glutamine or asparagine or both predominated, and in all species up to seven other amino acids were present in minor amounts. Small amounts of what appeared to be a peptide were also present in a number of species.

In bleeding sap from a number of herbaceous species—pumpkin (104), cucumber (182), corn (4, 153), tomato (182)—much of the nitrogen is present as amino acid nitrogen, glutamine being the predominant compound in most species.

Xylem sap from some woody species had other organic nitrogenous compounds as the major sap constituents (30, 33). Citrulline was found in sap from some species and allantoin or allantoic acid or both were found in others. Citrulline was also found in bleeding sap from members of the Betulaceae (157) and allantoic acid in bleeding sap from *Aesculus* (157). Mothes & Engelbrecht (133), on the basis of bleeding sap analyses, claimed that in *Acer* spp. allantoin and allantoic acid are of major importance in the translocation of nitrogen. The metabolic roles of these compounds have been recently reviewed (35). It is sufficient to note here that they all provide efficient forms of nitrogen transport and are all soluble nitrogen-rich compounds with this nitrogen being readily available for further metabolism.

Other nitrogenous compounds may also be involved in the transport of nitrogen in certain species. For example, γ-methylene glutamine is the predominant nitrogenous compound in bleeding sap from peanuts (82).

Many alkaloids are now known to be synthesized in the root system and

transported to the shoot through the xylem. For example, nicotine has been detected in bleeding sap from tobacco plants (68) and scopolamine in the sap from various species (135). More recently, paper chromatography of bleeding sap of tobacco (186) has revealed at least eight pyridine alkaloids of which only nicotine, nornicotine, and anabasine have been identified.

Translocation of Phosphorus and Sulphur

Information abount the form in which phosphorus is transported in the xylem is very limited. Phosphorus was found to be present in both organic and inorganic form in varying proportions in bleeding sap from squash and corn (146), but other workers (153) could find only inorganic phosphorus in bleeding sap from corn roots. No examination of the phosphorus in vacuum-extracted xylem sap has yet been made.

In bleeding sap collected from a number of species which had been growing in culture solution containing ^{32}P, three radioactive compounds were detected by chromatography (179). One of these was inorganic phosphate and the other was identified as phosphoryl choline (116). Of the ^{32}P in the bleeding sap, up to 6 per cent in barley and up to 20 per cent in tomato was present as this compound. This might be taken as an indication that some phosphorus is transported through the plants as phosphoryl choline; but in later work, not yet fully reported (111, 178), choline, added to the culture solution in which plants were growing, increased the phosphoryl choline in the roots but decreased phosphorus transport to the shoot.

When sulphate labelled with ^{35}S was fed to a number of species, the only radioactive spot in chromatograms of bleeding sap was inorganic sulphate (179). On the other hand, methionine and a peptide which yields cysteine have been found in xylem sap extracted from a number of species (33). In bleeding sap from corn up to 6 per cent of the sulphur was in a reduced form of which methionine and glutathione were the only compounds identified (78, 144).

Translocation of Iron

Immobil'zation of iron in the vascular system.—In some plants it has been observed that chlorotic symptoms apparently attributable to iron deficiency (i.e., which can be remedied by suitable application of iron) were not always accompanied by a shortage of iron in affected tissues (139). Olsen (138) was the first to offer an explanation of this apparent anomaly. He suggested that when iron is taken up from neutral or alkaline solutions it can be precipitated as ferric phosphate in the vascular bundles along the veins of a leaf. Thus, an analysis of this plant material might show apparently adequate iron present but much of it might not have left the vascular system. On roots of bean plants grown in culture solutions containing a high ratio of phosphorus to iron, Biddulph & Woodbridge (15) were able to demonstrate a precipitate containing both elements and they suggested that such precipitates might also occur in the vascular system.

Further support for Olsen's suggestion comes from the work of Rediske & Biddulph (154). Bean plants were grown in culture solution with a low

iron content. When the solution was at pH 7 and the phosphate concentration 0.001 *M*, chlorotic plants were produced. When radioactive iron was added to this culture medium little uptake could be detected. With a phosphate concentration of 0.0001 *M* and at pH 7, the leaves were mainly chlorotic but were markedly green along the veins. Autoradiographs of these plants showed a heavy deposition of iron along the veins, while the rest of the leaf blade remained relatively free of this element. When the phosphate concentration was 0.0001 *M* and the solution pH was 4, normal green leaves were produced and autoradiographs showed a rapid uptake and uniform distribution of iron in the leaf blade. Rediske & Biddulph suggest that under the first conditions the combination of pH and phosphate concentration in the external medium was such that iron uptake by the plants was prevented, but in the third set of conditions adequate iron uptake occurred. Where chlorotic leaves with green veins were produced Rediske & Biddulph suggest that the phosphorus concentration was low enough to allow the absorption of iron at pH 7.0 but phosphorus uptake was still great enough to cause precipitation of iron in the veins.

By growing soybean plants in soil in vessels that allowed the roots to grow out of the soil and into a culture solution, Brown *et al.* (46) were able to study the effect of varying culture solution composition on uptake and translocation of iron. All iron taken up by the plant came from the soil because this element was not included in the culture solution. When roots emerging from the soil were either kept cut or kept in water, normal green foliage developed on the plants but if emerging roots were kept in certain culture solutions, completely chlorotic plants resulted. Unfortunately, the composition of the culture solutions was varied in such a way that the particular ions responsible cannot be exactly determined, but again a high phosphate content of the plant was associated with chlorosis. Chlorotic plants in these experiments had somewhat lower iron contents than green plants but it is unlikely that the levels were low enough to account for the chlorosis. By applying radioactive iron either to the soil or to the leaves of plants grown in the above experiments and following its movements through the plant, it was shown that the mobility of this element was much less in high-phosphate plants.

It has not been possible to distinguish between failure of the plant to take up iron and the immobilization of iron in the tissues for many of the experiments reported. All iron-deficiency chlorosis in plants is certainly not due to immobilization of iron in the vascular system. In the experiments of Rediske & Biddulph mentioned above, plants grown in culture solutions with high phosphate apparently took up little iron. Brown *et al.* (44) have described two varieties of soybean differing markedly in their ability to obtain enough iron from certain soils. Reciprocal grafting of tops and roots of the two varieties showed that the variety used for the root system determined whether plants were chlorotic or not. Greater inactivation of iron did not appear to occur in the root system of the chlorosis-susceptible

variety so the difference between the two varieties seems to reside in a differing capacity for the absorption of iron.

Under certain conditions, however, it appears likely that in some plants immobilization of iron can occur in the vascular system to such an extent that the adjacent tissues become markedly deficient in iron. There also appears to be a direct relationship between the phosphate level in the plant and such immobilization. The effect of external factors such as pH of culture solution may operate by affecting relative uptake of iron and other ions such as phosphate.

Uptake and translocation of chelated iron.—It is known that iron is readily available to plants when supplied in culture solution as a chelated complex with ethylenediaminetetraacetic acid (EDTA) (96). Iron-deficiency chlorosis frequently can be remedied by the application of chelated iron to the affected plants (169), where the application of iron salts would give uncertain results. It is also known that some chelating agents added to the soil will allow sufficient iron to be taken up by plants that could not otherwise obtain it. These practical results have stimulated investigation of the role of chelating compounds in the uptake and translocation of iron in plants.

On the basis of the ratio of citric acid to iron needed in the culture solution for optimal plant growth, Bitcover & Sieling (20) had earlier suggested that iron might be taken up by the plant as a ferric organic complex ion rather than simply as either the ferric or ferrous ion.

The first indication that EDTA-Fe complexes could also be absorbed by the plant came from work with isotopically labelled EDTA. When corn seedlings were supplied with ^{15}N-EDTA-Fe (189) or orange seedlings with ^{14}C-EDTA-Fe (110) through the roots, either isotope could be detected throughout the plant. EDTA is very resistant to attack by soil microorganisms (169) and it seems unlikely that this compound was broken down before absorption. Subsequently, EDTA has been detected chemically throughout avocado plants (190) and in root exudate from excised roots of bean plants (87) when EDTA-Fe was supplied to the soil or culture solution, respectively.

Analyses of avocado leaves for both EDTA and iron after root feeding with EDTA-Fe suggested that both were taken up from the soil in equivalent amounts (190). There was also good agreement between the actual iron contents of various parts of the plant and the expected iron content calculated from the ^{15}N content after feeding with ^{15}N-EDTA-Fe for four days, indicating that EDTA and iron were probably translocated together through the plant. When soybeans were fed ^{14}C-labelled diethylenetriamine pentaacetic acid (DTPA)-Fe complex, the carbon label was found evenly distributed through the plant (91). On the other hand, after DTPA-^{55}Fe was added to soil the iron was found concentrated in the leaf and flower primordia, suggesting that the iron and chelate molecule are soon separated in the plant.

The ability of plants to absorb and transport certain chelating agents was demonstrated in experiments where sunflower seedlings were grown with their roots divided so that half were in a culture solution containing ferrous sulphate (196). If the other half of the roots was kept in the same culture solution but containing no iron, chlorotic plants resulted. However, if this second half was in a culture solution containing EDTA but no iron the plants were green. Weinstein *et al.* (196) suggest that the iron becomes inactivated in the roots immersed in the iron-containing solution. If the other roots can absorb a suitable chelating agent, which can be translocated, then this inactivated iron can be made available once again and becomes freely mobile through the plant.

The apparent ability of synthetic chelating agents to carry iron into and through plants led Stewart & Leonard (169) to suggest that chelates could be the natural means whereby iron was absorbed by plants. Support for this idea has also come from studies on the uptake of iron by varieties of soybean differing in susceptibility to iron chlorosis (45, 46). Under unfavourable conditions, such as high phosphate content, the susceptible variety appears less able to keep the iron in its tissues mobile. They suggest that some type of natural compound may function as a chelating agent and keep iron mobile. The fact that plant species and varieties differ in their ability to absorb and translocate iron suggests that they may differ in the quantity or quality of such natural chelating agents.

No suggestion that elements other than iron may be immobilized in the xylem appears to have been made. Iron deficiency is exceptional among known deficiencies of essential elements in that it is difficult to remedy with inorganic salts of iron. It is known that chelated forms of other elements can be readily absorbed by plants (187) but the role of possible natural chelating agents in the uptake of these elements has not been investigated.

TRANSLOCATION OF COMPLEX ORGANIC COMPOUNDS

In recent years during screening trials for possible systemic insecticides, systemic fungicides, herbicides, and plant growth regulators many synthetic chemicals or antibiotics produced by microorganisms have been applied to plants and there is now ample evidence that plants can take up many foreign complex organic molecules and that these can be transported through most of the plant body. The only compounds of interest to us here are those which have been shown to move in the xylem. In Table I are listed compounds for which there is some evidence of their movement in the xylem after absorption. The list is by no means exhaustive, e.g., Crowdy & Wain (64) and Fawcett *et al.* (76, 77), describe tests with a large range of aryloxyalkanecarboxylic and arylthioalkanecarboxylic acids many of which probably move in the xylem. The list gives, however, an indication of the wide range of foreign molecules which plants can take up and transport.

In most experiments of this type detection of the compound in the upper parts of the plant after its addition to the root environment has depended upon some form of bioassay, though in many cases compounds labelled with

TABLE I

ORGANIC COMPOUNDS WHICH MOVE IN THE XYLEM

Compound	Authority	Compound	Authority
Growth Regulators:	Authority	*Dimethyl carbomethoxypropenyl phosphate	(54)
Indoleacetic acid	(80, 88, 165)	*Demeton	(1, 126, 177)
Indolebutyric acid	(80, 88)	*Diethyl diethylaminoethyl thiophosphate	(127)
Indolepropionic acid	(88)	Herbicides:	
Naphthalene acetic acid	(80, 88)	*Aminotriazole	(57, 86, 122, 152, 162)
Phenylacetic acid	(88)	*Propham	(8)
Phenylpropionic acid	(88)	*Monuron	(57, 85, 129, 136, 137)
2,4-D	(57, 80, 130, 194, 197)	Trichloroacetic acid	(21)
2,4,5-T	(57)	Ammonium sulphamate	(52)
Antibiotics:		Maleic hydrazide	(57)
Streptomycin	(2, 58, 73, 131, 147, 148)	3-Hydroxy-1,2,4-triazole	(122)
Chlortetracycline	(22, 100)	*Chlorbis (ethylamino) triazine	(67)
Griseofulvin	(39, 40, 58, 59, 60, 171)	Sulphonamides:	
Penicillin	(199, 200)	Sulphanilamide	(61)
Chloramphenicol	(58, 59, 100, 147, 148)	Sulphacetamide	(61)
Cycloheximide	(192)	Sulphaguanidine	(61)
Oxytetracycline	(100)	Sulphapyridine	(61)
Systemic Insecticides:		Sulphadiazine	(61)
Bis (dimethylamino) fluorophosphine oxide	(65, 117)	Sulphathiazole	(61)
Sodium fluoroacetate	(66)	4:4′-Diaminodiphenylsulphone	(61)
*Schradan	(66, 95, 125, 159, 195, 205)		
*Paraoxon	(66)		
*Parathion	(167, 168, 205)		
*Diethyl chlorovinyl phosphate	(54)		

* Indicates name of compound as defined by Martin (118).

radioactive elements have been used. The appearance of biological activity or radioactivity in the upper parts of plants does not mean that the supplied compounds themselves have been absorbed and translocated. In some cases, e.g., streptomycin (147) and cycloheximide (192), compounds have been detected in leaves of plants with similar chromatographic properties to those originally supplied. In comparatively few cases has there been an actual demonstration that the supplied compound occurred unchanged in the foliage, but this has been done for griseofulvin, chloramphenicol (59, 60), and diethyl diethylaminoethyl thiophosphate (127). It has been shown that metabolism of the added compound did occur in other instances. Crowdy & Jones (62) have shown that some acetylation of sulphanilamide occurred in the roots before this compound was translocated, while Metcalf *et al.* (126), with demeton, and Racusen (152), with aminotriazole, using labelled materials, have both demonstrated derived compounds in foliage.

Rapid upward movement and distribution of compounds through the plant after their application to the medium in which roots were growing has often been taken to indicate that such compounds were transported

upwards through the plant in the transpiration stream. The complex relationship between ion uptake and water absorption by the root has already been discussed. However, if the uptake and distribution through the plant of a particular compound is varied directly by changes in transpiration rate then it seems reasonable to assume that the compound moves in the transpiration stream. Such evidence has been given on occasion (57, 60, 61, 88, 129, 136, 171). The presence of foreign compounds in guttation drops from leaves (40, 171, 199, 200) or in xylem exudates from excised roots (147, 165) of treated plants gives another basis for suggesting xylem movement of compounds. More direct proof of xylem transport has been provided with some compounds by ringing and xylem removal experiments. When a portion of the plant stem is killed by heat, the upward movement of a number of synthetic growth regulators is little affected (80, 88, 130, 165, 194, 197). Ringing of plants had no effect on the upward movement of demeton (1, 177) and monuron (136) while removal of a short length of xylem completely inhibited it.

Most of the foregoing discussion has concerned experiments where the foreign compounds were applied to the medium in which plant roots were growing. Many experiments have also been carried out where similar compounds have been applied to the leaves. Absorption of compounds applied in this way often occurs readily, but some compounds that are readily absorbed through roots can apparently also be taken up through the leaves only at a much slower rate [e.g., trichloroacetic acid (21)], or not at all [e.g., monuron (57, 85) and propham (8)].

The absorption of nutrients (201) and systemic insecticides (12) after foliar application has been reviewed recently. Little is known, however, of the particular vascular tissues involved in the transport of such compounds away from the foliage; but some indications exist that from the leaf surface some such compounds move first into the phloem. For example, more demeton was found in stem phloem than xylem after application to leaves (177) and it has been shown (176) that movement of schradan away from apple leaves was prevented by stem ringing. Crafts & Yamaguchi (57) have followed the uptake and distribution of a number of ^{14}C-labelled compounds (2,4-D, aminotriazole, maleic hydrazide, and monuron), all of which were taken up readily through roots. When these compounds were applied to the leaves of the same test plants, marked differences in uptake and distribution were noted: maleic hydrazide and aminotriazole were readily absorbed and were highly mobile through the plant, 2,4-D was absorbed and moved out of the treated leaf but its movement was more restricted, while monuron apparently would not move at all. On the basis of autoradiographic studies, Crafts & Yamaguchi believe that the immobility of monuron is due to its inability to enter the phloem. 2,4-D can enter the phloem readily but its mobility is restricted because it is readily accumulated by other cells as it moves in the phloem. Both maleic hydrazide and aminotriazole show no such tendency to be accumulated and thus move freely in the phloem. Both compounds can move into the xylem from the

phloem to a varying extent and can then circulate around the plant.

Even with compounds readily taken up through the root system, final distribution of a compound within the plant can be very uneven. There is often a tendency for some systemic insecticides supplied in this way to be located first in the younger and more active tissues (159, 195) and some compounds move more readily into terminal leaves (125). Within leaves these compounds tend to accumulate around the periphery (126, 177).

Differences occur between plant species in regard to uptake and transport of foreign compounds. Cucumber seedlings will readily take up both streptomycin and chloramphenicol from solution (147). With time, the level of streptomycin in leaves increased to a level greater than that in the nutrient solution, while chloramphenicol remained at a lower level. Tomato and broad bean will also take up chloramphenicol and transport it through the plant (58). Tomato will take up streptomycin but tends to hold it in the lower parts of the plant, while broad beans will not take up any streptomycin.

Some of these anomalies may be explicable on the basis of the suggestion made by Charles (53) that xylem elements may be negatively charged. He was able to demonstrate rapid petiolar uptake of acid fuchsin working with excised leaves, while uptake of the basic dyes, methylene blue and safranin, was much slower. Analogous results were obtained with the acidic antibiotic, penicillin, and the basic antibiotic, streptomycin. If the xylem elements were negatively charged, then retarding of the entry and movement of the positively charged basic organic molecules might be expected. Charles has suggested a mechanism whereby the walls of xylem vessels could become negatively charged but no further evidence appears to have been offered. Crowdy & Pramer (63) have pointed out that the readily translocated antibiotics (chloramphenicol, penicillin, and griseofulvin) are all neutral or acidic compounds, whereas anomalous behaviour has been reported for the basic (streptomycin and neomycin) and amphoteric (chlortetracycline and oxytetracycline) compounds. Pramer (148) and Dye (73) have suggested that the slow movement of streptomycin in some plants may thus be explained by its positive charge, while chloramphenicol, a neutral compound, might not be expected to be held up in the xylem. However, the difference in behaviour of streptomycin in cucumbers, broad beans, and tomatoes remains unexplained. Pramer has shown that streptomycin was actively accumulated by *Nitella* cells (149) but chloramphenicol entered by simple diffusion (150). It may be that differences in accumulating ability also play some part in these higher plant differences.

Because there is little concrete evidence to the contrary, plants should not be thought capable of taking up most molecules with which they are presented. Much of the work mentioned has been concerned with the development of systemic insecticides, fungicides, etc., and only those compounds which have emerged successfully from earlier screening trials have received detailed study. Many compounds may have been eliminated in such trials because they are not taken up or not transported by plants, but there is often no way of knowing this. Thus, Pramer (147) could demonstrate no

uptake of neomycin, oxytetracycline, or chlortetracycline by cucumber seedlings, but because his only criterion of uptake was the appearance of antibiotic activity in the leaves he could not decide whether these compounds had not been absorbed or had been inactivated following absorption.

Work with these complex organic compounds, while carried out with other objectives, has illustrated some of the adaptability or perhaps lack of discrimination of the transport mechanisms of plants. This work may not yet have contributed much to our understanding of uptake and transport of normal plant metabolites, but it may be that future studies with selected compounds of varying molecular size, shape, and charge may assist in defining the capabilities of transport mechanisms in plants.

MOVEMENT OF NUTRIENTS OUT OF THE XYLEM

Nutrients may move from the transpiration stream to adjacent cells during passage up the xylem. Evidence for this type of movement from the xylem depends on xylem stripping experiments such as were used by Stout & Hoagland (172). In such experiments longitudinal strips of bark are carefully separated from the underlying xylem, both ends of the strips remaining attached. That such manipulations cause little upset to the plant can be demonstrated by binding the separated bark strips back into place; then the pattern of movement of radioisotopes is indistinguishable from that in intact plants. If waxed paper is inserted between bark and xylem before rebinding, any movement of solutes between these tissues in this region should be prevented. Using radioactive isotopes of potassium, sodium, phosphorus, and bromine supplied to plant root systems, Stout & Hoagland showed that the tracer ions found in bark in their intact plants must have moved there from the adjacent xylem tissues as no tracer was found in the bark when the two tissues were separated by waxed paper. Skoog (165) had earlier obtained evidence for the lateral movement of auxin from the transpiration stream and more recently Tietz (177) showed that the systemic insecticide, demeton, will move laterally from xylem to phloem.

It is thus clear that nutrients can move directly from the transpiration stream to adjacent bark tissues but it is not yet possible to assess the quantitative importance of such movement. The data of Stout & Hoagland showed that substantial transfer from the xylem can occur throughout the length of the stem. Autoradiographic studies (16), however, have shown that the highest concentrations of an isotope, fed through the roots of young plants, are to be found first in the leaves and stem apices. This indicates that the bulk of the nutrients were probably carried through to the termini of the xylem system.

Little is known of the movement of nutrients from the endings of xylem elements. The vein endings in leaves frequently consist of one, or a few tracheids, surrounded by a sheath of parenchyma cells (75). The walls and contents of these sheath cells show many of the staining reactions of endodermis cells but there appears to be no evidence to suggest what role,

if any, is played by these cells in the loss of nutrients from the termini of the vascular system.

REDISTRIBUTION OF NUTRIENTS WITHIN THE PLANT

The amount of nutrients reaching particular plant organs through the xylem should be influenced by the amount of water lost from that particular organ. For this reason it might be thought that in mature leaves, where transpiration is high, the greatest accumulation of nutrients might occur. Biddulph (14), however, has shown that while radioactive phosphate will move readily into mature leaves, nevertheless concentrations there will remain less than those in the younger tissues of the apical meristem where transpiration rates might be expected to be considerably lower.

The possibility of nutrients being exported from the leaves to other parts of the plant has been investigated by Biddulph. When radioactive phosphate was injected into bean leaves, export down the petiole could soon be detected (13) and it was shown that such movement was in the phloem (18). Autoradiographs have demonstrated (16, 17) the movement of radioactive phosphate from older leaves to younger expanding leaves and meristematic areas. When fed to roots of rapidly growing seedlings both ^{32}P and ^{35}S were initially concentrated in the first leaves, but as these matured both elements moved to new leaves (16). Biddulph (17) regards this export of nutrients from leaves as a mechanism that comes into play only when the level of transpiration results in more nutrients being supplied to particular leaves from the transpiration stream than is required by their metabolism, but with leaves of certain ages this is probably the normal occurrence. Evaluation of the normal extent of this export of nutrients in leaves of particular ages must await comparison of transpiration rates with isotope accumulation.

Agreement that most upward movement of nutrients from the root occurs in the xylem is not inconsistent with the idea that considerable upward movement can occur in the phloem. There is agreement that redistribution through the plant of nutrients from the termini of the xylem system takes place in the phloem. The direction in which this occurs will be largely influenced by the relative utilization of particular nutrients in different parts of the plant.

The idea of soluble materials circulating more or less freely within plants is not a new one (16) but there has been little evidence for such circulation until recently. Older ideas considered xylem and phloem as separate tissues, with little thought being given to the possibility of exchange between them. Evidence for loss of nutrients from the transpiration stream to the phloem and for the re-export in the phloem of nutrients brought to leaves in the transpiration stream has already been discussed. There is now also evidence that direct movement of solutes from phloem to xylem can occur. By combining leaf injection and bark stripping experiments, Biddulph & Markle (18) were able to show that movement of ^{32}P from injected leaves was primarily in the phloem but lateral movement

into the xylem also occurred. Confirmatory results were obtained when both radioactive sulphur and phosphorus were supplied to leaves, and their distribution studied in sections of stem tissue by autoradiography (19). All the requirements for circulation of nutrients in plants now appear to be demonstrated. Solutes delivered to leaves can be re-exported in the phloem to other parts of the plant or they can pass from the phloem to the xylem where they can again be carried up in the transpiration stream.

Although movement of substances from the phloem to xylem undoubtedly occurs, it is clear that there is not free movement of all substances from the phloem. For instance, sugars are undoubtedly transported in the phloem but are scarcely found in the xylem sap (29).

Experiments on circulation in plants have been mainly short-term ones with rapidly growing seedlings, but in studies with sugar cane (48) it was shown that circulation of some elements continued for an extended period. Phosphorus and nitrogen could be detected in the extensive new growth of this species one and three months, respectively, after labelled compounds were first applied.

Perhaps all elements should not be expected to circulate equally freely within plants. Biddulph *et al.* have contrasted the behaviour of phosphorus, sulphur, and calcium in bean plants (16). Plants were placed with their roots in solutions containing radioisotopes for an hour and then transferred to nonradioactive solutions. The redistribution of each element was followed by radioautography during the next 96 hr. of growth. Phosphorus remained mobile, the concentration of isotope being highest in the youngest leaves and stem apices throughout the period of the experiment. Sulphur was initially freely mobile but became fixed in young leaves, while calcium apparently did not move from the leaves where it was first deposited.

Calcium has come to be regarded as the most immobile of all the essential elements (16, 160). Once delivered to a particular organ by the transpiration stream it apparently cannot recirculate. Calcium, however, cannot be completely immobile since movement of ^{45}Ca to newly formed buds of pine trees has been detected up to two seasons after trunk injection (79). With respect to mobility the nonessential elements, strontium (155) and yttrium (156), behave like calcium.

Though most elements taken up through the root system of plants are probably transported in the xylem, yet autoradiographic studies on plants fed with a number of isotopes have revealed different distribution patterns for each element (108). Certain elements, such as zinc, show a tendency to concentrate in the vascular strands of the petiole and leaf veins (158, 193). Other elements, such as molybdenum, become more evenly distributed through the leaf tissue with little remaining in the veins. Chlorine, known to be freely mobile in some plants (203), tends to concentrate in leaf veins although it also spreads throughout the leaf blades (180). Cobalt tends to concentrate along the margins of leaves and there is evidence that this element can be lost entirely from the leaf by washing (107).

The factors that determine the particular distribution of each element

are not known. Little is known about the effects of the concentration of any element, in either the plant or the culture solution, on the movement of that element within the plant. Greater mobility of injected iron was observed when tissue concentrations of this element were low (154) and this might also be true for other elements. On the other hand, the mobility of an element must also depend on the nature of the compounds into which it is incorporated. The effects of chelating agents on iron distribution in plants suggest that perhaps these or other organic compounds may also affect the mobility and distribution of other elements.

LITERATURE CITED

1. Ahmed, M. K., Newsom, L. D., Roussel, J. S., and Emerson, R. B., *J. Econ. Entomol.*, **47**, 684–91 (1954)
2. Anderson, H. W., and Nienow, I., *Phytopathology*, **37**, 1 (1947)
3. Anderssen, F. G., *Plant Physiol.*, **4**, 459–76 (1929)
4. Andreeva, I. N., *Fiziol. Rasteny* (in English translation) **4**, 508–17 (1957)
5. Arisz, W. H., *Koninkl. Ned. Akad. Wetenschap. Proc.*, **48**, 420–46 (1945)
6. Arisz, W. H., *Ann. Rev. Plant Physiol.*, **3**, 109–30 (1952)
7. Arisz, W. H., Helder, R. J., and van Nie, R., *J. Exptl. Botany*, **2**, 257–97 (1951)
8. Baldwin, R. E., Freed, V. H., and Fang, S. C., *J. Agr. Food Chem.*, **2**, 428–30 (1954)
9. Bathurst, N. O., and Mitchell, K. J., *New Zealand J. Agr. Research*, **1**, 540–52 (1958)
10. Bennett, J. P., Anderssen, F. G., and Milad, Y., *New Phytologist*, **26**, 316–23 (1927)
11. Bennett, J. P., and Oserkowsky, J., *Am. J. Botany*, **20**, 632–37 (1933)
12. Bennett, S. H., *Ann. Rev. Entomol.*, **2**, 279–96 (1957)
13. Biddulph, O., *Am. J. Botany*, **28**, 348–54 (1941)
14. Biddulph, O., in *Mineral Nutrition of Plants*, 269–75 (Truog, E., Ed., University of Wisconsin Press, Madison, Wis., 469 pp., 1951)
15. Biddulph, O., and Woodbridge, C. G., *Plant Physiol.*, **27**, 431–44 (1952)
16. Biddulph, O., Biddulph, S. F., Cory, R., and Koontz, H., *Plant Physiol.*, **33**, 293–300 (1958)
17. Biddulph, O., Biddulph, S. F., and Cory, R., in *Radioisotopes in Scientific Research*, **4**, 340–50; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
18. Biddulph, O., and Markle, J., *Am. J. Botany*, **31**, 65–71 (1944)
19. Biddulph, S. F., *Am. J. Botany*, **43**, 143–8 (1956)
20. Bitcover, E. H., and Sieling, D. H., *Plant Physiol.*, **26**, 290–303 (1951)
21. Blanchard, F. A., *Weeds*, **3**, 274–8 (1954)
22. Blanchard, F. A., and Diller, V. M., *Am. J. Botany*, **38**, 111–12 (1951)
23. Bois, E., and Chubb, W. O., *Can. J. Research*, **20B**, 114–20 (1942)
24. Bois, E., Dugal, L. C., and Lessard, M., *Can. J. Research*, **20B**, 1–4 (1942)
25. Bois, E., and Nadeau, A., *Can. J. Research*, **14B**, 373–80 (1936)
26. Bois, E., and Nadeau, A., *Can. J. Research*, **16B**, 114–20 (1938)
27. Bois, E., and Nadeau, A., *Can. J. Research*, **16B**, 121–33 (1938)
28. Bollard, E. G., *Nature*, **171**, 571 (1953)

29. Bollard, E. G., *J. Exptl. Botany*, **4**, 363–68 (1953)
30. Bollard, E. G., *Nature*, **178**, 1189–90 (1956)
31. Bollard, E. G., *Australian J. Biol. Sci.*, **10**, 279–87 (1957)
32. Bollard, E. G., *Australian J. Biol. Sci.*, **10**, 288–91 (1957)
33. Bollard, E. G., *Australian J. Biol. Sci.*, **10**, 292–301 (1957)
34. Bollard, E. G., in *The Physiology of Forest Trees*, 83–93 (Thimann, K. V., Ed., The Ronald Press Company, New York, N.Y., 678 pp., 1958)
35. Bollard, E. G., *Symposia Soc. Exptl. Biol.*, **13**, 304–29 (1959)
36. Bond, G., *J. Exptl. Botany*, **7**, 387–94 (1956)
37. Bonner, J., *Science*, **129**, 447–50 (1959)
38. Breazeale, E. L., McGeorge, W. T., and Breazeale, J. F., *Plant Physiol.*, **25**, 413–19 (1950)
39. Brian, P. W., *Ann. Appl. Biol.*, **39**, 434–38 (1952)
40. Brian, P. W., Wright, J. M., Stubbs, J., and Wey, A. M., *Nature*, **167**, 347–49 (1951)
41. Briggs, G. E., and Robertson, R. N., *Ann. Rev. Plant Physiol.*, **8**, 11–30 (1957)
42. Brouwer, R., *Acta Botan. Neerl.*, **3**, 264–312 (1954)
43. Brouwer, R., *Acta Botan. Neerl.*, **5**, 287–314 (1956)
44. Brown, J. C., Holmes, R. S., and Tiffin, L. O., *Soil Sci.*, **86**, 75–82 (1958)
45. Brown, J. C., Holmes, R. S., and Tiffin, L. O., *Soil Sci. Soc. Am., Proc.*, **23**, 231–4 (1959)
46. Brown, J. C., Tiffin, L. O., Holmes, R. S., Specht, A. W., and Resnicky, J. W., *Soil Sci.*, **87**, 89–94 (1959)
47. Broyer, T. C., and Hoagland, D. R., *Am. J. Botany*, **30**, 261–73 (1943)
48. Burr, G. O., Hartt, C. E., Tanimoto, T., Takahashi, D., and Brodie, H. W., in *Radioisotopes in Scientific Research*, **4**, 351–68; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
49. Burstrom, H., *Physiol. Plantarum*, **1**, 124–35 (1948)
50. Burstrom, H., and Krogh, A., *Svensk Botan. Tidskr.*, **41**, 17–44 (1947)
51. Canning, R. E., and Kramer, P. J., *Am. J. Botany*, **45**, 378–82 (1958)
52. Carvell, K. L., *Forest Sci.*, **1**, 41–3 (1955)
53. Charles, A., *Nature*, **171**, 435–6 (1953)
54. Corey, R. A., Dorman, S. C., Hall, W. E., and Glover, L. C., *J. Econ. Entomol.*, **46**, 386–87 (1953)
55. Crafts, A. S., *Plant Physiol.*, **11**, 63–79 (1936)
56. Crafts, A. S., and Broyer, T. C., *Am. J. Botany*, **25**, 529–35 (1938)
57. Crafts, A. S., and Yamaguchi, S., *Hilgardia*, **27**, 421–54 (1958)
58. Crowdy, S. H., *Ann. Appl. Biol.*, **45**, 208–15 (1957)
59. Crowdy, S. H., Gardner, D., Grove, J. F., and Pramer, D., *J. Exptl. Botany*, **6**, 371–83 (1955)
60. Crowdy, S. H., Grove, J. F., Hemming, H. G., and Robinson, K. C., *J. Exptl. Botany*, **7**, 42–64 (1956)
61. Crowdy, S. H., and Jones, D. R., *J. Exptl. Botany*, **7**, 335–46 (1956)
62. Crowdy, S. H., and Jones, D. R., *J. Exptl. Botany*, **9**, 220–28 (1958)
63. Crowdy, S. H., and Pramer, D., *Chem. & Ind. (London)*, **6**, 160–62 (1955)
64. Crowdy, S. H., and Wain, R. L., *Ann. Appl. Biol.*, **38**, 318–33 (1951)
65. David, W. A. L., *Ann. Appl. Biol.*, **39**, 203–10 (1952)

66. David, W. A. L., and Gardiner, B. O. C., *Ann. Appl. Biol.*, **38**, 91–110 (1951)
67. Davis, D. E., Funderburk, H. H., and Sansing, N. G., *Weeds*, **7**, 300–9 (1959)
68. Dawson, R. F., *Am. J. Botany*, **29**, 66–71 (1942)
69. Dimbleby, G. W., *Plant and Soil*, **4**, 141–53 (1952)
70. Dimond, A. E., *Ann. Rev. Plant Physiol.*, **6**, 329–50 (1955)
71. Dixon, H. H., and Atkins, W. R. G., *Sci. Proc. Roy. Dublin Soc.*, **14**, 374–92 (1915)
72. Dixon, H. H., and Atkins, W. R. G., *Sci. Proc. Roy. Dublin Soc.*, **15**, 51–62 (1916)
73. Dye, M. H., *Ann. Appl. Biol.*, **44**, 567–75 (1956)
74. Eaton, F. M., *Am. J. Botany*, **30**, 663–74 (1943)
75. Esau, K., *Plant Anatomy*, 420–32 (John Wiley and Sons, Inc., New York, N.Y., 1953)
76. Fawcett, C. H., Spencer, D. M., and Wain, R. L., *Ann. Appl. Biol.*, **43**, 553–68 (1955)
77. Fawcett, C. H., Spencer, D. M., and Wain, R. L., *Ann. Appl. Biol.*, **45**, 158–76 (1957)
78. Fejér, D., *Acta Biol. Acad. Sci. Hung.*, **9**, 159–79 (1958)
79. Ferrell, W. K., and Johnson, F. D., *Science*, **124**, 364–65 (1956)
80. Ferri, M. G., *Contribs. Boyce Thompson Inst.*, **14**, 51–68 (1945)
81. Findlay, G. H., and Snell, J. F., *Can. J. Research*, **13B**, 269–75 (1935)
82. Fowden, L., *Ann. Botany* (*London*), **18**, 417–40 (1954)
83. Greenridge, K. N. H., *Ann. Rev. Plant Physiol.*, **8**, 237–56 (1957)
84. Grossenbacher, K. A., *Am. J. Botany*, **26**, 107–9 (1939)
85. Haun, J. R., and Peterson, J. H., *Weeds*, **3**, 177–87 (1954)
86. Hauser, E. W., and Thompson, J., *J. Agr. Food Chem.*, **2**, 680–81 (1954)
87. Heinonen, S., and Waris, H., *Physiol. Plantarum*, **9**, 618–23 (1956)
88. Hitchcock, A. E., and Zimmermann, P. W., *Contribs. Boyce Thompson Inst.*, **7**, 447–76 (1935)
89. Hoagland, D. R., and Broyer, T. C., *Plant Physiol.*, **11**, 471–507 (1936)
90. Holgate, K. C., *Changes in the Composition of Maple Sap during the Tapping Season* (Bull. 742, N.Y. [Geneva] Agr. Expt. Station, 14 pp., 1950)
91. Holmes, R. S., and Brown, J. C., *Soil Sci.*, **80**, 167–79 (1955)
92. Hylmö, B., *Physiol. Plantarum*, **6**, 333–405 (1953)
93. Hylmö, B., *Physiol. Plantarum*, **8**, 433–49 (1955)
94. Hylmö, B., *Physiol. Plantarum*, **11**, 382–400 (1958)
95. Ivy, E. E., Iglinsky, W., and Rainwater, C. F., *J. Econ. Entomol.*, **43**, 620–26 (1950)
96. Jacobson, L., *Plant Physiol.*, **26**, 411–13 (1951)
97. Jacobson, L., Hannapel, R. J., and Moore, D. P., *Plant Physiol.*, **33**, 278–82 (1958)
98. James, W. O., and Baker, H., *New Phytologist*, **32**, 317–43 (1933)
99. Johnson, L. P. V., *Can. J. Research*, **23C**, 192–97 (1945)
100. Klemmer, H. W., Riker, A. J., and Allen, O. N., *Phytopathology*, **45**, 618–25 (1955)
101. Kramer, P. J., *Plant and Soil Water Relationships*, 175–76 (McGraw-Hill Book Company, Inc., New York, N.Y., 1949)
102. Kramer, P. J., in *Encyclopedia of Plant Physiology*, **II**, 290–315 (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, 1072 pp., 1956)

103. Kramer, P. J., *Science,* **125,** 633–5 (1957)
104. Kulaeva, O. N., Silina, E. I., and Kursanov, A. L., *Fiziol. Rasteny* (in English translation), **4,** 497–504 (1957)
105. Kursanov, A. L., in *Radioisotopes in Scientific Research,* **4,** 494–509; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
106. Ladefoged, K., *Plant and Soil,* **1,** 127–34 (1948)
107. Langston, R., *Proc. Am. Soc. Hort. Sci.,* **68,** 366–69 (1956)
108. Langston, R., *Proc. Am. Soc. Hort. Sci.,* **68,** 370–76 (1956)
109. Lauritzen, C. W., *J. Am. Soc. Agron.,* **26,** 807–19 (1934)
110. Leonard, C. D. and Stewart, I., *Proc. Am. Soc. Hort. Sci.,* **62,** 103–10 (1953)
111. Loewenberg, J. R. and Tolbert, N. E., *Plant Physiol.,* **33,** Suppl., xx (1958)
112. Lowry, M. W., Huggins, W. C., and Forrest, L. A., *The Effect of Soil Treatment on the Mineral Composition of Exuded Maize Sap at Different Stages of Development* (Bull. 193, Georgia Agr. Expt. Station, 28 pp., 1936)
113. Lowry, M. W. and Tabor, P., *Science,* **73,** 453 (1931)
114. Lundegårdh, H., *Arkiv för Botanik,* **32A** (12) 1–139 (1945)
115. Lundegårdh, H., *Physiol. Plantarum,* **3,** 103–51 (1950)
116. Maizel, J. V., Benson, A. A., and Tolbert, N. E., *Plant Physiol.,* **31,** 407–8 (1956)
117. Martin, H., *Ann. Appl. Biol.,* **36,** 153–55 (1949)
118. Martin H., *Guide to the Chemicals Used in Crop Protection* 3rd ed. (Canada Department of Agriculture, 306 pp., 1957)
119. Marvin, J. W., in *The Physiology of Forest Trees,* 95–124 (Thimann, K. V., Ed., The Ronald Press Company, New York, N.Y., 678 pp., 1958)
120. Mason, T. G., and Maskell, E. J., *Ann. Botany (London),* **42,** 189–253 (1928)
121. Mason, T. G., and Phillis, E., *Botan. Rev.,* **3,** 47–71 (1937)
122. Massini, P., *Acta Botan. Neerl.,* **7,** 524–30 (1958)
123. Meeuse, B. J. D., *New Phytologist,* **48,** 125–42 (1949)
124. Meeuse, B. J. D., *Acta Botan. Neerl.,* **1,** 216–21 (1952)
125. Metcalf, R. L., and March, R. B., *J. Econ. Entomol.,* **45,** 988–97 (1952)
126. Metcalf, R. L., March, R. B., Fukuto, T. R., and Maxon, M., *J. Econ. Entomol.,* **47,** 1045–55 (1954)
127. Metcalf, R. L., Stafford, E. M., Fukuto, T. R., and March, R. B., *J. Econ. Entomol.,* **50,** 205–10 (1957)
128. Miller, J. T., and Byers, H. G., *J. Agr. Research,* **55,** 59–68 (1937)
129. Minshall, W. H., *Can. J. Botany,* **32,** 795–98 (1954)
130. Mitchell, J. W., and Brown, J. W., *Botan. Gaz.,* **107,** 393–407 (1945)
131. Mitchell, J. W., Zaumeyer, W. J. and Anderson, W. P., *Science,* **115,** 114–15 (1952)
132. Mothes, K., *Die Kulturpflanze,* **1,** 157–69 (1953)
133. Mothes, K., and Engelbrecht, L., *Flora (Jena),* **139,** 586–97 (1952)
134. Mothes, K., and Kala, H. *Naturwissenschaften,* **42,** 159 (1955)
135. Mothes, K., Trefftz, G., Reuter, G., and Romeike, A., *Naturwissenschaften,* **41,** 530–31 (1954)
136. Muzik, T. J., Cruzado, H. J., and Loustalot, A. J., *Botan. Gaz.,* **116,** 65–73 (1954)
137. Muzik, T. J., Cruzado, H. J., and Morris, M. P., *Weeds,* **5,** 133–34 (1957)

138. Olsen, C., *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **21**, 15–63 (1935)
139. Oserkowsky, J., *Plant Physiol.*, **7**, 253–59 (1932)
140. Pierre, W. H., and Pohlmann, G. G., *J. Am. Soc. Agron.*, **25**, 144–60 (1933)
141. Pohlmann, G. G., and Pierre, W. H., *J. Am. Soc. Agron.*, **25**, 160-71 (1933)
142. Pollard, J. K., and Sproston, T., *Plant Physiol.*, **29**, 360–64 (1954)
143. Porter, H. K., and May, L. H., *J. Exptl. Botany*, **6**, 43-63 (1955)
144. Potapov, N. G., and Fejér, D., *Agrokémia és Talajtan*, **5**, 47–52 (1956)
145. Potapov, N. G., and Fejér, D., *Agrokémia és Talajtan*, **5**, 53–56 (1956)
146. Potapov, N. G., and Molnár-Keresztes, I., *Acta Botanica Acad. Sci. Hung.*, **2**, 171–82 (1955)
147. Pramer, D., *Ann. Appl. Biol.*, **40**, 617–22 (1953)
148. Pramer, D., *Ann. Botany (London)*, **18**, 463–70 (1954)
149. Pramer, D., *Arch. Biochem. and Biophys.*, **62**, 265–73 (1956)
150. Pramer, D., *Exptl. Cell Research*, **16**, 70–74 (1959)
151. Priestley, J. H., and Wormall, A., *New Phytologist*, **24**, 24–38 (1925)
152. Racusen, D., *Arch. Biochem. and Biophys.*, **74**, 106-13 (1958)
153. Ratner, E. I., and Samoilova, S. A., *Fiziol. Rasteny* (in English translation), **5**, 207–18 (1958)
154. Rediske, J. H., and Biddulph, O., *Plant Physiol.*, **28**, 576–93 (1953)
155. Rediske, J. H., and Selders, A. A., *Plant Physiol.*, **28**, 594–605 (1953)
156. Rediske, J. H., and Selders, A. A., *Am. J. Botany*, **41**, 238–42 (1954)
157. Reuter, G., and Wolffgang, H., *Flora (Jena)*, **142**, 146–55 (1954)
158. Riceman, D. S., and Jones, G. B., *Australian J. Agri. Research*, **9**, 730–44 (1958)
159. Ripper, W. E., Greenslade, R. M., and Hartley, G. S., *Bull. Entomol. Research*, **40**, 481–501 (1949)
160. Ririe, D., and Toth, S. J., *Soil Sci.*, **73**, 1-10 (1952)
161. Robertson, R. N., in *Encyclopedia of Plant Physiology*, **IV**, 243–79 (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, 1210 pp., 1958)
162. Rogers, B. J., *Weeds*, **5**, 5–11 (1957)
163. Russell, R. S., and Shorrocks, V. M., in *Radiosotopes in Scienctific Research*, **4**, 286–303; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergammon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
164. Scott, L. I., and Priestley, J. H., *New Phytologist*, **27**, 125–41 (1928)
165. Skoog, F., *Am. J. Botany*, **25**, 361–72 (1938)
166. Skoog, F., Broyer, T. C., and Grossenbacher, K. A., *Am. J. Botany*, **25**, 749-59 (1938)
167. Smith, F. F., and Clifford, P. A., *J. Econ. Entomol.*, **43**, 708–12 (1950)
168. Starnes, O., *J. Econ. Entomol.*, **43**, 338–42 (1950)
169. Stewart, I., and Leonard, C. D., in *Mineral Nutrition of Fruit Crops*, 775–80 (Childers, N. F., Ed., Rutgers University Press, New Brunswick, N.J., 907 pp., 1954)
170. Stocking, C. R., in *Encyclopedia of Plant Physiology*, **III**, 489–502 (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, 1074 pp., 1956)
171. Stokes, A., *Plant and Soil*, **5**, 132–42 (1954)
172. Stout, P. R., and Hoagland, D. R., *Am. J. Botany*, **26**, 320–24 (1939)
173. Sutcliffe, J. F., *Biol. Revs. Cambridge Phil. Soc.*, **34**, 159–220 (1959)
174. Swanson, C. A., *Ohio J. Sci.*, **57**, 331–36 (1957)

175. Thomas, W., *Science*, **66**, 115–16 (1927)
176. Thomas, W. D. E., and Bennett, S. H., *Ann. Appl. Biol.*, **41**, 501–19 (1954)
177. Tietz, H., *Höfchen-Briefe*, **7**, 1–55 (1954)
178. Tolbert, N. E., and Loewenberg, J. R., *Federation Proc.*, **17**, 323 (1958)
179. Tolbert, N. E., and Wiebe, H., *Plant Physiol.*, **30**, 499–504 (1955)
180. Toth, S. J., and Kretschmer, A. E., *Soil Sci.*, **77**, 293–302 (1954)
181. van Andel, O. M., *Acta Botan. Neerl.*, **2**, 445–521 (1953)
182. van Die, J., *Koninkl. Ned. Akad. Wetenschap. Proc.*, **C61**, 572–78 (1958)
183. van Die, J., *Koninkl. Ned. Akad. Wetenschap. Proc.*, **C62**, 50–58 (1959)
184. van Nie, R. Helder, R. J., and Arisz, W. H., *Koninkl. Ned. Akad. Wetenschap. Proc.*, **53**, 567–75 (1950)
185. Van Overbeek, J., *Am. J. Botany*, **29**, 677–83 (1942)
186. Wada, E., Kisaki, T., and Ihida, M., *Arch. Biochem. Biophys.*, **80**, 258–67 (1959)
187. Wallace, A., in *Symposium on the Use of Metal Chelates in Plant Nutrition*, 4–23 (Wallace, A., Ed., The National Press, Palo Alto, Calif., 80 pp., 1956)
188. Wallace, A., and Mueller, R. T., in *Tree Physiology Studies at U.C.L.A., Special Report No. 1*, 139–43 (Wallace, A., Ed., University of California, Los Angeles, 178 pp., 1958)
189. Wallace, A., and North, C. P., *Calif. Agr.*, **7** (8) 10 (1953)
190. Wallace, A., North, C. P., Mueller, R. T., Shannon, L. M., and Hemaidan, N., *Proc. Am. Soc. Hort. Sci.*, **65**, 9–16 (1955)
191. Wallace, A., Zidan, Z. I., Mueller, R. T., and North, C. P., *Proc. Am. Soc. Hort. Sci.*, **64**, 87–104 (1954)
192. Wallen, V. R., and Millar, R. L., *Phytopathology*, **47**, 291–94 (1957)
193. Wallihan, E. F., and Heymann-Herschberg, L., *Plant Physiol.*, **31**, 294–99 (1956)
194. Weaver, R. J., and DeRose, H. R., *Botan. Gaz.*, **107**, 509–21 (1946)
195. Wedding, R. T., and Metcalf, R. L., *Botan. Gaz.*, **114**, 180–89 (1952)
196. Weinstein, L. H., Purvis, E. R., Meiss, A. N., and Uhler, R. L., *J. Agr. Food Chem.*, **2**, 421–25 (1954)
197. Weintraub, R. L., and Brown, J. W., *Plant Physiol.*, **25**, 140–49 (1950)
198. Wiebe, H. H., and Kramer, P. J., *Plant Physiol.*, **29**, 342–48 (1954)
199. Winter, A. G., and Willeke, L., *Naturwissenschaften*, **38**, 262–64 (1951)
200. Winter, A. G., and Willeke, L., *Naturwissenschaften*, **38**, 457 (1951)
201. Wittwer, S. H., and Teubner, F. G., *Ann. Rev. Plant Physiol.*, **10**, 13-32 (1959)
202. Wolffgang, H., and Mothes, K., *Naturwissenschaften*, **40**, 606 (1953)
203. Woolley, J. T., Broyer, T. C., and Johnson, G. V., *Plant Physiol.*, **33**, 1–7 (1958)
204. Wormall, A., *Biochem. J.*, **18**, 1187–202 (1924)
205. Zeid, M. M. I., and Cutkamp, L. K., *J. Econ. Entomol.*, **44**, 898–905 (1951)

TRANSPORT IN THE PHLOEM[1,2]

By Martin H. Zimmermann

Maria Moors Cabot Foundation for Botanical Research, Harvard University
Petersham, Massachusetts

The manuscript of this article was prepared for press just after the 9th International Botanical Congress in Montreal, during which three half-days were devoted to problems of translocation. These discussions were most stimulating although perhaps not every aspect of the problem was equally well covered. Since most of the data presented at the Congress are still unpublished, only a few of the Congress reports are reviewed in this article which covers primarily the literature since the review of Esau *et al.* (33). Occasional reference is made to older papers, either to relate current developments to past progress or to focus the reader's attention on older, little known foreign literature.

Anatomical Aspects

Resch (94, 95) studied the development of the phloem of a number of plant species. Isolated sieve tubes are described; these occur interfascicularly, singly or in groups, and are connected to the bundles. Cheadle & Esau (14) presented an extensive report on the secondary phloem of the Calycanthaceae. They found most of the sieve elements to have one or two (occasionally up to five) ontogenetically associated companion cells. Of the 193 critically examined sieve elements, however, nine had no associated companion cells. The authors stress that the occasional absence of companion cells should be taken into account in any concept of the role of these cells in the functioning of sieve elements. Braun (9) investigated the nodal phloem of the Dioscoreaceae and found the sieve-tube continuity there to be interrupted by a group of cells (*Phloembeckenzellen*) rich in plasma and differing from parenchyma cells by the absence of starch.

Esau & Cheadle (31) reported a study of wall thickening in sieve elements, the so-called nacreous thickening, that often gives the sieve elements a glistening appearance in fresh sections. In microchemical tests the nacreous thickening showed a positive reaction for cellulose and no indication of lignification. Of the 142 species investigated, 45 showed this thickening in various degrees, from a barely perceptible layer to one that almost occludes the lumen. In another paper the same authors report on a survey of sizes of sieve pores and their contents (callose cylinder and connecting strand) (32). Pore diameters in the 160 investigated species ranged from a fraction of a micron to about 14 μ. In the same plant the pores were sig-

[1] The survey of literature pertaining to this review was concluded August 1959.

[2] The following abbreviations are used: THO (tritium-labeled water); 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).

nificantly larger in the sieve plates (end walls) than in lateral sieve areas. The percentage of transverse cell area occupied by pores ranged from 83.2 per cent (connecting strands 49.3 per cent) in sieve elements with simple sieve plates, to 112.9 per cent (strands 32.0 per cent) in elements with oblique scalariform plates.

The submicroscopic phloem structure has been the subject of several studies. Frey-Wyssling & Müller (41) described the differentiation of the skeleton of *Cucurbita* sieve plates. Fibril deposition in local fields of transverse walls is early discontinued and tangential strands are deposited around these places. The original network finally disappears and there are no membranes across the sieve plates in mature sieve tubes. This is still the case when whole sieve tubes are studied instead of the skeleton only, according to Beer (3). Furthermore, this author reported the presence of some vesicles in mature *Cucurbita* sieve tubes, but a lack of mitochondria, endoplasmic reticulum, and nucleus. In contrast to these findings, Schumacher & Kollmann (104) reported plastids, mitochondria, and membrane systems in the cytoplasm of the sieve cells of *Passiflora coerulea*. These authors also claim to have demonstrated the tonoplast in the sieve cells. Plasmodesmata, according to Beer (3), are highly developed between companion cells and sieve tubes. Killing and fixation of tissues is of crucial importance for structural studies at submicroscopic dimensions and the authors are aware of the possibility that their electron micrographs may yet be improved.

An extensive review on callose has been presented by Eschrich (34). He stressed the importance of taking into consideration the rapid callus formation after tissue injury. He described the work of Fischer (37) who found, in 1886, that tissue, killed in intact plants by hot liquids, showed only traces of callose on the sieve plates, whereas after conventional chemical fixation there was an abundance of it. A similar observation has been made in our own laboratory. During the summer months, when the sieve tubes are conducting, no callose is found on the sieve plates if the bark of trees is sectioned fresh immediately. After storage of the stem for a few hours, or after fixation of fresh bark tissue in the conventional formaldehyde-alcohol-acetic acid mixture, there are appreciable callose masses on most sieve plates. Eschrich (35) described callus formation even in epidermal cells of *Allium cepa* after 1.5 hr. of plasmolysis in fructose. A detailed study of callose beads isolated from *Vitis vinifera* by Kessler (59) has added to our knowledge of the physical properties of this substance. Hydrolysis of the methylated compound and enzymatic degradation indicated a β-1:3 linkage of the glucose units.

An interesting discussion in the literature concerns the interpretation of plasmolysis of open (severed) cells (28,121). Although the experiments do not concern phloem cells, they are nevertheless worth considering in the interpretation of sieve-element plasmolysis.

Identity of Translocated Substances

Methods.—If an incision is made into the inner bark of certain trees, one can obtain an exudate from the sieve tubes of the phloem. This was first described 100 years ago by Hartig (47) who had, 23 years earlier, discovered the sieve tubes (46). Evidence that the exudate comes from the sieve tubes and does represent translocated material has been reviewed elsewhere (134, 135). Although sieve-tube exudation has been demonstrated in a large number of plant species (17, 47, 79, 87, 128, 134), its use as a method for studies of translocation is, for the present at least, more or less restricted to woody dicotyledons. It is particularly suitable for quantitative studies with large trees. It may be of interest to note that sieve-tube exudate was collected by man long before its botanical discovery. Huber has described a small industry in southern Italy still concerned with the cultivation of certain varieties of *Fraxinus* for the commercial production of sieve-tube sugar [(55); see also the literature cited therein].

Aphids and coccids are among the numerous parasites on the translocation channels. It has been shown for a large number of aphids that their stylet tips are inserted into the sieve tubes of the phloem [cf. (75) and the citations therein]. Although they are called "sucking" insects, sieve-tube turgor is of considerable assistance to the insect during the feeding process. This is also indicated by the observation of Mothes & Engelbrecht (78) of continued exudation after withdrawal of the stylet bundle. Mittler (75 to 77) has developed a method for obtaining exudate from stylet stumps, using the lachnid *Tuberolachnus salignus*. These relatively large aphids colonize mainly four-year-old stems of *Salix* sp. The aphids were usually left on the plant for 2 to 3 hr. until they had secreted several droplets of "honeydew," the sugary excretion product typical of many aphids and coccids. They were then anaesthesized in a gentle stream of carbon dioxide and cut from their stylets with a fine splinter of a razor blade. The exudate was collected, the stylet stumps being sleeved within the meniscus of a capillary (76). It is interesting to note that feeding aphids that were subjected to carbon dioxide anaesthesia for 24 hr. did not exude honeydew. When their stylets were severed during this period, however, sap exuded readily from the stylet stumps (75). This is further evidence in support of the previously expressed view that stylet sap is exuded by the forces within the plant.

The aphid-stylet method is unsurpassed in many respects. With it one can obtain an exudate uncontaminated by traces of substances from the cut surface of the outer bark. Furthermore, the flow is from one single file of sieve elements. On the other hand, quantitative short-time experiments are nearly impossible to carry out because the rate of flow from one stylet is only 1 to 2 $mm.^3$ per hour. Quantitative studies are further complicated by the possibility that the concentration of stylet exudate is lower than that of the moving solution. Zimmermann (135, 136) found that there was os-

motic dilution of the exudate (obtained with Hartig's method) from a single tapping cut. Within the first 15 min. the concentration of the exudate had dropped to about 70 per cent of its initial value. If the same phenomenon occurs with stylet exudate, then the published concentration (around 10 per cent w/v) is lower than that of the moving solution. This point has to be cleared up before the method can be applied to certain quantitative studies.

The methods of obtaining translocated material described above take advantage of our knowledge of the conducting tissue and its high longitudinal conductibility. Another method of identifying translocated material is the displacement of translocates by labeled substances of the same type. $C^{14}O_2$ is usually supplied to one leaf and various plant parts are subsequently analyzed for radioactive substances. A method for the local application of $C^{14}O_2$ to a small leaf area has been described by Aronoff (1). Analyses after displacement are valuable for translocation studies only if experimental times are kept short in order to minimize nonmoving products (metabolites) which appear after the translocates have reached their destination.

Carbohydrates.—The bulk of the translocated material in the phloem, about nine-tenths or more, consists of carbohydrates. This can easily be visualized if one considers the material needed for growth of a potato, a tree trunk, or a cotton hair. It is more difficult to visualize in the case of, for example, a lemon fruit with a high acid content. Yet, grafting experiments of Erickson (30) showed that lemon fruits have their own metabolism which is independent of the leaves from which they are fed. Immature "sour" lemon fruits grafted on "sour" or "sweet" lemon plants developed the same high acid content, while "sweet" fruits also developed as such, whether they grew on "sweet" or "sour" plants.

Wanner (119) and Ziegler (128) reported paper chromatographic analyses of sieve-tube exudates from a dozen European trees. The only sugar found was sucrose. Hexoses and hexose phosphates were absent. Zimmermann (134) tested exudates of 16 North American tree species. He found sucrose to be predominant and the only sugar in a number of species. However, besides sucrose, some trees carried sugars of the raffinose family of oligosaccharides, namely raffinose, stachyose, and verbascose. These sugars consist of sucrose with one or more D-galactose units attached. Traces of raffinose occurred in a number of species; appreciable amounts of raffinose and stachyose were found in white ash (*Fraxinus americana*), elm (*Ulmus americana*), and linden (*Tilia americana*). Oligosaccharides of the raffinose family (including sucrose) have also been found in sieve-tube exudates of a number of tropical trees. Hexoses were not found in any. Besides the sugars, however, the sieve-tube exudate of *Fraxinus* and *Syringa* (Oleaceae), and *Terminalia* (Combretaceae) species contains mannitol, a sugar alcohol, in appreciable amounts [(134, 136); and unpublished results].

Sugars of the raffinose family of oligosaccharides are not restricted to exudates. Pristupa (89) reported recently that $C^{14}O_2$ supplied to leaves of

Cucurbita pepo, moved out predominantly in the form of stachyose and verbascose.

Exudate from aphid stylets on *Salix* sp. (53, 76, 77,) *Heracleum Mantegazzianum,* and *Picea abies* (131) normally contained sucrose as the only sugar. Weatherley *et al.* (122) reported occasional traces of raffinose (up to 0.4 per cent) in addition to sucrose in stylet sap of *Salix.*

All reports concerning sieve-tube exudates, covering a total of about 45 species, stress the complete absence of hexose even in chromatographically detectable traces (53, 76, 119, 122, 128, 131, 134). Reports on displacement of naturally occurring translocates with $C^{14}O_2$-labeled photosynthates agree with these findings to such an extent that it seems certain that sucrose or oligosaccharides are the predominant translocated sugars (6, 65, 68, 89, 113, 118). Besides sucrose, however, glucose and fructose have been reported in amounts that seem to depend largely on experimental procedures and times. As experimental times are shortened, the label decreases in hexoses. It must be noted that it is difficult with this method to discriminate between actually moving substances and those that have become stationary. Some light is shed on this last point by the experiments of Swanson (112) and Swanson & El-Shishiny (113) with $C^{14}O_2$ supplied to a leaf of the grapevine (*Vitis labruscana* var. Concord). Several interesting results were obtained. First, the ratio of labeled glucose/labeled fructose was very nearly one everywhere in the bark. This was not so for the unlabeled sugars. Second, the hexose/sucrose ratio was much lower for radioactive than for nonradioactive sugars. Third, the labeled hexose/labeled sucrose ratio decreased continuously from 0.083 at 8.8 cm. to 0.037 at 115.6 cm. from the supply leaf. These results suggest strongly that sucrose is the only translocated sugar in the grape, and that the hexoses are secondary products, derived from hydrolysis after sucrose has left the actual translocational channels. These conclusions are supported by the previously reviewed results of sieve-tube exudate analyses.

An interesting approach to the same problem has been reported by Kursanov *et al.* (65). These investigators double grafted Jerusalem artichoke/sunflower/Jerusalem artichoke, and sunflower/Jerusalem artichoke/sunflower. Upon feeding $C^{14}O_2$ to one of the upper leaves, the C^{14} was found to move down the stem mainly in the form of sucrose (80 to 85 per cent of the label). The ratio, labeled hexose/labeled sucrose, decreased along the stem and did not change while passing from one plant species to the other, although the extrafascicular metabolism is different in the two species (predominance of monosaccharides in the cortex of sunflower, predominance of fructosan-type storage oligosaccharides in the cortex of Jerusalem artichoke).

Nitrogenous compounds.—Amino acids and amides are known to be translocated mainly out of aging leaves and flowers. This makes the direct displacement method, which is valuable in the case of sugars, difficult if

not impossible to apply. Recent reports mainly concern exudate analyses. Mittler (74, 76) found aspartic acid, glutamic acid, serine, threonine, alanine, valine, leucine and/or isoleucine, phenylalanine, asparagine, glutamine, and possibly γ-amino-butyric acid in the stylet sap of aphids that had been feeding on willow stems.

Of particular interest are the seasonal changes in the nitrogen concentration of the stylet exudate of willows. Immediately after the termination of the plant's dormancy when bud swelling was prominent, the total nitrogen concentration was 0.2 per cent (w/v), during bud burst and appearance of the first leaves it was 0.12 per cent, during leaf maturity 0.03 per cent, and during leaf senescence 0.13 per cent. Such an increase in nitrogenous matter was also found by Hill (53) and Weatherley *et al.* (122) after the plants had been transferred to the dark. It is obviously caused by the removal of protein nitrogen from the leaves prior to abscission. Essentially the same concentration changes of nitrogenous matter have been reported by Zimmermann (136) for the sieve-tube exudate of white ash. Ziegler (128) too, found a two- to tenfold increase in amino acids and amides in sieve-tube exudates of trees during autumnal leaf senescence. During the summer he found in *Quercus borealis, Q. robur, Q. petraea,* and *Acer platanoides* glutamic acid and aspartic acid, and, in smaller amounts, alanine (total amount 0.4 to 0.5 mg./ml.). In the fall, the concentration of these substances increased, and others, like asparagine, glutamine, serine, methionine, valine, and leucine, also appeared to make up a total concentration of 2 to 4 mg./ml. In *Robinia pseudo-acacia* about a dozen compounds were present during the summer (total concentration 0.7 to 0.8 mg./ml.); most of them, in particular, proline, increased in the fall. This increase in nitrogen content during natural as well as induced leaf senescence is further evidence that exudates represent translocates. Ziegler (128) determined protein nitrogen (trichloroacetic acid-insoluble N) in sieve-tube exudates. Concentrations ranged from about 0.1 (*Robinia*) to about 1 mg./ml. (*Quercus*) with no longitudinal gradient along the trunk nor with seasonal changes. These were considered nonmobile substances (cytoplasm), torn out during the tapping process. The proportion of these nonmoving components in exudates is much higher in cucurbits (18, 135) than in trees.

Other substances.—The concentration of the sugars in sieve-tube exudates is usually between 10 and 25 per cent (w/v), that of amino acids and amides between 0.03 and 0.4 per cent (w/v). The concentration of other substances is usually even lower. At this level it is difficult to decide whether a substance in the exudate is really a translocate, or if it is "torn-out" from the sieve tubes, or if it is from the cut surface of the outer bark. The aphid-stylet method would probably be ideal for such analyses, but it has not yet been applied extensively. Ziegler (128) found inorganic phosphate in exudates of a number of trees in concentrations below 1 mg./ml. Bound phosphates (phosphatides, etc.) were below 0.1 mg./ml. total. He considered the latter nonmobile.

Movement of Applied Substances

General aspects.—The largest number of papers in the past few years dealt with the translocation of applied substances. Many substances, applied to mature leaves and various other plant parts, will be taken up and translocated through the phloem together with the assimilate stream, regardless of whether they are naturally occurring or synthetic compounds. This phenomenon is of considerable practical importance from two entirely opposite points of view: the distribution of foliar applied plant nutrients and the distribution of pesticides.

The substances have been administered to the plants in many different ways: by dipping the cut petiole, or a cut flap of a leaf, or a whole leaf into the solution; and by painting or spraying the solution onto the leaves, etc. Crafts (19) emphasized that such methods tend to cause a local increase of the hydrostatic pressure within the apoplast (the water-conducting system: xylem and cell walls), particularly under conditions of active transpiration and if the plant has been injured. The resulting outward movement via the xylem to other plant parts has to be discriminated from phloem transport.

Furthermore, it is important to distinguish between uptake into the sieve tubes and transport through these channels. The complexity of the processes here included in the gross term "uptake" should not be underestimated. There are several steps between the absorption of molecules from a solution and their final secretion into the sieve tubes (19). Some of these steps require metabolic energy. This has been shown in many ways and with a wide variety of plants. The last step, secretion into the sieve tubes, occurs against a concentration gradient (24, 56, 98, 122). This is not only true for sugars, but also, particularly in the fall, for nitrogen and phosphorus compounds (128).

A number of investigators reported sugar transformation during uptake. Pennell & Weatherley (86) found sucrose uptake into leaf disks of *Atropa belladonna* under aerobic conditions to be four times as intense as under anaerobic conditions, the decrease caused by anaerobiosis being reversible. Hay & Thimann (52) reported that 2,4-D is taken up and translocated with the assimilate stream from a mature leaf, either in the light, or in the dark if an energy source such as sucrose is provided. Mannitol, arabinose, and urea, not representing sources of metabolic energy, cannot replace sucrose. A similar observation has been made by Barrier & Loomis (2) for P^{32}. These authors used detached leaves in order to exclude translocation. They found ready absorption of P^{32} in both light and dark; however, in the presence of either light or sugars there was, with time, a distinct accumulation of the radioactivity in the veins. Loomis called this accumulation, which is different from absorption, "loading." It represents the last steps of uptake and is equivalent to secretion into the sieve tubes.

Early phases of uptake have been investigated microscopically by Schumacher and co-workers (102, 105, 106). Droplets of solutions placed on leaf surfaces of various plants stimulated the number of plasmodesmata of the

outer epidermis ("ectodesmata") depending on the type and the concentration of the solution.

Uptake itself represents a certain amount of "transport," namely the movement from the applied solution or cells where photosynthesis takes place to the sieve tubes of the phloem. The words transport and translocation are used in this paper, however, exclusively for the long distance movement in the sieve tubes, except of course, where otherwise stated (e.g., xylem transport).

Uptake and translocation of sugars.—Simple sugars that are not easily metabolized by the plant may be taken up and translocated as such. This has been shown for arabinose and sorbose (133), and for galactose (128). Sugars that are easily metabolized undergo transformation during uptake, although a residual amount may move as such. After applying an equimolar mixture of galactose and fructose (or glucose) to the inner bark of *Robinia Pseudo-acacia,* the sieve-tube exudate from a point below the place of application contained, besides sucrose, either galactose alone or galactose plus a smaller amount of fructose (128). Putman & Hassid (93) infiltrated leaf disks of *Canna indica* with radioactive fructose, glucose, and fructose-labeled sucrose. They found in all three cases a rapid conversion to sucrose of which both hexose moieties were labeled. Kursanov & co-workers (33) found the same for the uptake of radioactive sugars into sugar beet leaves. The conducting bundles, subsequently isolated, contained sucrose. Upon hydrolysis, this yielded radioactive fructose and glucose in comparable amounts. It would be of extreme interest for the plant physiologist to be able to introduce, for instance, galactose-labeled raffinose into the sieve tubes of plants. However, for the present at least, such an experiment is not possible because of sugar transformation during uptake.

Nelson & Gorham (80) applied radioactive sugars to a primary leaf of bean seedlings for 20 min. in the light and subsequently transferred some of the plants to the dark for 14 hr., while the others remained in the light for the same length of time. Although some of the sugars were lost in the dark, presumably by respiration, more activity was transported out of the leaf in the dark than in the light. This could be explained in various ways. It may mean that uptake of photosynthates into the sieve tubes has priority over uptake from an applied solution. Mere absorption into the leaf blade may have taken place in these experiments during the first 20 min. In another experiment the same authors dipped the cut petiole into glucose-C^{14}, fructose-C^{14}, and sucrose-C^{14} solutions for 3 to 15 min. (81). While the outward movement of glucose and fructose was unaffected by a steam girdle (movement via nonliving tissue) the sucrose movement was completely stopped by such treatment.

It has been found by Gauch *et al.* (43, 107) that boron somehow plays a role in the translocation of sugars. These authors came to this conclusion not only through their own experimental results but also from a critical

review of the literature (44): boron-deficient plants appear to suffer from a lack of translocation of sugars from leaves. They suggested at first that sugars may form an ionizable sugar-boric acid complex which may move across membranes more readily than nonborated, nonionized sugar molecules. In a later paper they suggest that boron might affect sugar translocation indirectly (29). They found that boron depresses the rate of conversion of glucose-1-phosphate to starch by starch phosphorylase. In the presence of boron, more glucose-1-phosphate could be available for other reactions, such as sucrose synthesis, and thus result in an increase in translocation from the site of synthesis to other plant parts. Skok (108), taking up the same subject, reached the conclusion that the apparent effect of boron on sugar translocation could be related to the effect of boron on cellular activity. Ziegler (128) analyzed sieve-tube exudates of a number of tree species and found no boron (sensitivity of the method 5 γ/ml.). The effect of boron on the translocation of sugars, therefore, seems to be an indirect one.

Uptake and translocation of minerals.—Absorption and transport of a number of foliar-applied radioactive isotopes has been studied by Bukovac & Wittwer (10). Using recovery in nontreated plant parts as the criterion, they found Rb, Na, and K most readily absorbed and most highly mobile; P, Cl, S, Zn, Cu, Mn, Fe, and Mo were intermediate with decreasing mobility in the order given; while Ca, Sr, and Ba were absorbed into the leaf but not exported. The order of this list may be subject to certain adjustments depending on the molecular types in which the isotopes are applied. Koontz & Biddulph (60) made such a study with labeled phosphorous in various molecular types.

The mobility of foliar-applied nutrients is dependent upon a number of factors (2, 7, 11, 45, 60, 114 to 116, 123). Although these factors are of practical importance, most of them affect uptake into the sieve tubes or into the mesophyll rather than the process of translocation itself. Two examples, in addition to what has already been said, may illustrate this. Calcium is considered highly immobile in the phloem. Bukovac *et al.* (11) found that if bean leaves are treated with $Ca^{45}Cl_2$ and subsequently anesthetized with diethyl ether, Ca will easily move out of the treated leaves into other plant parts. The uptake mechanism may have been altered here, and perhaps any dominating side-track into calcium storage was blocked. Or, Ca may have been lost to the apoplast upon anaesthesia and carried out via the transpiration stream. Gustafson (45) studied the absorption and translocation of $Co^{60}Cl_2$. Transport stopped in the dark, was resumed upon application of sugars, but not with mannitol or glycine [cf. the findings of Hay & Thimann (52) above]. Although absorption had taken place in the dark, the lack of transport must have been caused by the lack of uptake into the sieve tubes. This can easily be visualized if one considers that phloem translocation in a tree takes place under the dead bark in complete darkness. Darkening of the petiole and stem of a seedling, surely, would not itself affect phloem translocation.

Uptake and translocation of herbicides.—As in the case of foliar-applied nutrients, it is essential to know optimal conditions for uptake and translocation in order to insure maximal distribution within the plant. A number of papers take up this subject. It has been shown for potassium and phosphorus that a nutritional deficiency may be a limiting factor (97, 99). Temperature, formulation, the use of surfactants, place of application, etc., are other important factors (2, 20, 52, 70, 127).

Crafts *et al.* (19, 85), working with radioactive 2,4-D and tracing its movement by autoradiography, pointed out that some killing and drying methods, and especially pressing, may allow a redistribution within the plant after termination of the actual experiment. Freeze-drying proved most reliable. A detailed description of various techniques is given in a later paper by Yamaguchi & Crafts (127). Experiments with a number of plant species indicated that C^{14} applied as 2,4-D always moved together with the assimilate stream, not against it (19, 20, 22, 71). For instance, there was no movement out of very young expanding leaves, but movement into them from older, expanded leaves. Movement was mainly downward from lower leaves, mainly upward from upper leaves, and in both directions to a comparable extent from leaves in between. Depending on the growth of the plant, movement was at times predominantly downward into roots, at other times predominantly into growing shoots, or flowers, or fruits, etc. (19, 20, 71). In comparative tests on the uptake and distribution of various labeled herbicides it was found that the movement of 2,4-D and 2,4,5-T was restricted by their accumulaton into living cells of the leaves, and their movement was apparently limited to phloem. Aminotriazole and maleic hydrazide moved in both phloem and xylem, whereas dimethylurea did not enter the phloem but was readily mobile in the xylem. Phloem-mobile compounds did not move out of chlorotic leaves (22).

It is, of course, not certain that C^{14}, pictured on autoradiographs, is still in the form of the applied substance, especially if long times are involved. This difficulty was avoided by Hay & Thimann (49 to 52) who followed 2,4-D and 2,4,5-T movement by subsequent extraction from plant parts and bioassay. It was found with this method that much of the 2,4-D was metabolized in the plant. In bean seedlings this loss was considerably faster in light than in darkness, but even in the dark, three-quarters of the applied 2,4-D disappeared in five days (51). Although transport was completely inhibited by ringing, the 2,4-D was found about equally distributed between xylem and cortex. This shows its ready removal from the phloem. None of the herbicide ever entered the roots (52). The same poor phloem mobility was found with marabú (*Dichrostachys nutans*), a woody plant native to Africa that covers vast areas of valuable land in Cuba in a dense jungle. The truly spectacular effectiveness of 2,4-D treatment, in this case, stems from the running down of the solution along the outside of the roots in the case of basal sprays and to distribution in the xylem in the case of cut-stump treatments (49, 50).

Direction and Polarity of Movement

Direction.—In general, the direction of movement is from source to sink, e.g., from old leaves to very young, actively growing parts, to roots, etc. This, already well known, was confirmed many times during recent years. There is no movement out into the parenchyma of darkened parts of mature leaves (1,4) since darkened leaf parenchyma does not represent a sink. Investigations by Nelson & Gorham (80) indicate more upward movement into the growing stem tip from a primary leaf in the light and more downward movement into the roots in the dark. A more intense downward movement of photosynthates toward the roots during the night has also been found by Pristupa & Kursanov (90). In another report, Nelson & Gorham (82) describe the effect of the roots on the translocation of radioactive amino acids and amides after dipping the cut petiole into solutions. Phloem movement seems to have played only a secondary role in these experiments since most substances moved past steam girdles.

Novitsky (84) studied phloem translocation from leaves to shoots in a short- (red perilla, *Perilla nankinensis*) and a long-day plant (lettuce) under flower-inducing and noninducing daylengths. During the first 2 to 3 weeks after transferring the plants from a noninducing to an inducing photoperiod a smaller percentage of C^{14} moved out of the leaf than in other plants which remained under the noninducing photoperiod. In spite of this, under a flower-inducing daylength a greater part of the outflowing C^{14} entered the roots than under the noninducing photoperiod. The author discusses the possible explanations of these results. Chailakhyan & Butenko (13) studied the translocation of the flowering stimulus in the same short-day plant (*Perilla nankinensis*). They maintained the plants under a long day but bagged some of the leaves to induce flowering on certain parts of the plant. At the same time they applied $C^{14}O_2$ to a particular leaf. Extensive experimental combinations showed that the flowering stimulus was always translocated together with the assimilate stream.

Lateral transport in the phloem is known to be very slight. Caldwell (12), to mention just one example, described experiments in which he obtained asymmetric growth of stem and capitulum by unilateral defoliation. Prokofyev *et al.* (91, 92) found that each leaf of a sunflower plant supplies a curved radial series of seeds in the head. This was detected by autoradiography after painting certain leaves repeatedly with labeled acetate, phosphates, etc. In the case of external disturbance, a certain lateral transfer was found possible. Removal of leaves from one orthostichy did not affect the normal formation of flower head and seeds, so evidently neighboring leaves can supply flowers that have lost their "own" leaves. Removal of leaves from 3 to 4 orthostichies, however, resulted in asymmetric development of the flower head. Redistribution of phosphate within the flower head was absent.

Polarity of movement.—Sax (101) and Dickson & Samuels (26) induced phloem blocks in apple trees by inverting a ring of bark and found that P^{32}

applied as phosphate was accumulated at the ring. This raises the question of polar transport in the phloem. Is it impossible for photosynthates to move through an inverted section of phloem? Sax explains the blocking effect by the failure of renewal of phloem elements as a result of a lack of movement of auxins and nutrients into the inverted section. Lack of auxin movement is undoubtedly the primary effect here since auxins are known to be translocated in polar directions.

Phloem movement may be reversed, in time. For instance, in the petioles of very young leaves and in shoot tips movement is acropetal. The direction of movement is reversed, however, by the time the leaves have completed their growth. As far as is known, the sieve tubes as such do not exhibit a polarity of transport. This conclusion is reached in an older paper by Zimmerman & Connard (132), in which plants were grafted by the inarch method and later pruned to produce stems of M- and W-shape. To reach the roots, photosynthates had to move repeatedly upward and downward through the phloem. In other experiments shoots were caused to grow on overhanging portions of split stems. To reach the roots, photosynthates were forced to move upward on one side of the stem and downward on the other side. Movement was indicated by normally continuing growth. The same conclusion was reached by Weatherley *et al.* (122) on the basis of stylet exudation with phloem supply from either of the two directions.

Circulation of substances in plants.—A circulation of substances in plants may occur by subsequent, and perhaps repeated, downward movement in the phloem and upward movement in the xylem. Such a circulation on an annual basis was proposed for carboyhdrates around 1850 by Hartig, whose extensive investigations are summarized in his *Textbook for Foresters* (48). Other substances, particularly nitrogenous compounds, are known to be exported from aging leaves and other plant parts such as the petals of flowers, and imported into younger still growing parts. The literature covering this phenomenon has been reviewed by Fischer (38).

More recently, circulation has been shown to occur within short times and without intermediate storage. Biddulph *et al.* (5) determined the circulation patterns of P^{32}, S^{35}, and Ca^{45} in the bean plant by autoradiographic means from single aliquots of tracer administered to the roots during a 1 hr. period. The calcium showed its expected phloem immobility; it did not circulate after its initial delivery via the transpiration stream. A portion of the phosphorus displayed sustained circulation during the 96 hr. experimental period, whereas sulfur, originally mobile, was rapidly captured by the metabolism of the young leaves. Similar circulation patterns were reported for chlorine by Woolley *et al.* (126).

It is an old observation of horticulturists that trees may acquire an appearance of nitrogen deficiency when girdled. This peculiar observation has received considerable clarification during recent years. It has been found that most nitrogen moving upward through the xylem is in or-

ganic form (8, 88, 96, 125). Kursanov & co-workers (61, 63, 64, 66, 90) have studied the metabolism of roots for a number of years. They found that the upward-moving organic nitrogenous substances are synthesized in the roots from sugars (mainly sucrose) that come down from the leaves through the phloem and from mineral nitrogen that comes up from the soil. In 22-day-old pumpkin plants, for instance, they found that 18 to 50 per cent of the photosynthates exported from the leaves had entered the roots. Within 3 hrs., about 60 per cent (per cent radioactivity after photosynthesis in $C^{14}O_2$) of the sugar that had entered the roots was transformed into organic acids and amino acids. Mineral nutrition stimulated the flow of sugars toward the roots. On the other hand, nitrogen deficiency led to a sharp (but reversible) decrease of sugar transport toward the roots. Nearly one-half of the radioactivity that had entered the roots returned to the aerial organs via the xylem. The composition of nitrogenous compounds in the xylem is typical for certain plant groups. Citrulline, for example, is typical for the Betulaceae (96, 125). Amino acids are always present and inorganic nitrogen plays a very insignificant role (8).

Movement in opposite directions.—This point is of crucial importance in connection with possible mechanisms of phloem translocation. Movement in opposite directions at any one time and in any one channel has never been found. The results of most papers describing movement in opposite directions can be explained in terms of upward movement in the xylem and re-export from the leaves through the phloem. In the much cited experiments of Chen (15), for example, P^{32} that had been applied to lower leaves may have moved up in the transpiration stream and descended together with the labeled photosynthates through the phloem. In fact, the rather long experimental times of 12 to 17 hr. are ample to allow for circulation of label in the sense of Biddulph *et al.* (5) or Kursanov (64). Even if the xylem of a woody plant is removed, possible upward movement via the extrafascicular transpiration path should not be ignored. [For a discussion of the extrafascicular path of water see (110, 111) and the literature therein cited.]

Movement in a young plant is out of a mature leaf, downward to roots, and upward to the growing tip. This is not bidirectional movement in a sense incompatible with mass flow. If we consider two leaves on the same stem, one above the other, both with similar translocation patterns, bidirectional movement in the stem section between the leaves exists as far as the whole stem section is concerned. It has been reported above, however, that movement from leaves in intact plants is strictly longitudinal along the stem with every leaf having its own channel, so to speak. If we consider this finding, we cannot call the movement in the stem section between the two leaves bidirectional.

It may be of interest to recall a paper of Rouschal (100) at this point. Rouschal observed the transport of fluorescent dyes in the internal and external phloem of cucurbits. Movement in these two channels took place in

opposite directions at times, sometimes naturally, in other cases experimentally induced. Thus, an apparent bidirectional movement actually occurred here in two different channels within the same conducting bundle.

Metabolism and Translocation

Willenbrink (124), without severing the sieve tubes, exposed the central bundle of the petiole of *Pelargonium zonale* and enclosed it in a glass chamber that was to contain inhibitor substances. The rest of the plant remained intact. Movement of the fluorescent dye which was applied to the leaf blade was followed visually by dissection and microscopic observation after termination of the experiment. Evidence of nitrogen and phosphorus movement was obtained from analyses of small leaf samples before, during, and after the experiment. N and P were induced to move out of the leaf by darkening the leaf 2 to 4 days before the end of the experimental period (induced leaf senescence), a method first described by Schumacher in 1930 (103). Willenbrink displaced the air in the glass chambers with purified nitrogen, hydrogen, or carbon monoxide gas. Translocation was not affected in any case, even in experiments of over 24 hr. duration. These results seem to be in direct contradiction to those found by Curtis (23) and by Mason & Phillis (72). Curtis was unable to check translocation by coating the petiole with melted wax, but he did obtain indications of decreased translocation when he applied nitrogen gas under slight pressure. The overpressure in Curtis' experiments may have forced the nitrogen gas up through the petiolar cavity into the leaf blade where it could have interfered with the leaf metabolism. Mason & Phillis covered branches with plasticine. This may have had other effects upon the bark tissues, and in some of their experiments the plants were badly mutilated (three girdles, bark pried from wood, etc.).

In further experiments, Willenbrink (124) tested the effect of some respiratory inhibitors. HCN, vaporized from a $10^{-3}M$ solution, reversibly blocked the transport of fluoresceine, nitrogenous substances, phosphorus compounds, and labeled photosynthates. Irreversible inhibition of translocation was obtained with 2,4-dinitrophenol, arsenite, azide, and iodoacetic acid. Fluoride had but a slight effect. These results are similar to those of Kendall (58), but more extensive. Both authors are aware of the possibility of the inhibitors being carried, via the transpiration stream, up into the leaf where they could interfere with uptake. Willenbrink claims to have excluded this possibility by stating that the fluoresceine was taken up even under conditions of transport inhibition. However, this was a purely qualitative observation. Nevertheless, these experiments show that phloem translocation is dependent upon the metabolism of the tissue. We do not know which cells of the complex phloem tissue are involved nor if the inhibition directly, or indirectly, affects the process of translocation, for instance through weakening of the sieve-tube semipermeability. Any specific interpretation, at this point, would be little more than speculation.

Isolated conducting bundles have a respiration rate several times higher than that of surrounding tissue. This had been reported earlier (33, 62), and was confirmed by Willenbrink (124). Ziegler (130) found xylem and phloem to be equally responsible for the two- to threefold increase in respiration upon isolation from surrounding tissue. Even wood tissue of trees showed such an increase (conifers 1.1- to 1.7-fold, deciduous trees two- to twelvefold) (129). This, of course, makes R_Q values of isolated material quite meaningless. Ziegler concluded that the high respiration rate of isolated bundles has no direct relation to phloem transport. This view was supported by his observation that isolated bundles (whole bundles or phloem only) are unable to conduct faster than the same tissue after having been killed in liquid nitrogen (130). Interruption of the sieve tubes seems to disturb translocation irreversibly.

Kursanov *et al.* (67) studied the glycolytic enzymes of isolated conducting tissues of sugar beet. They found hexokinase, phosphohexoisomerase, and aldolase but no phosphoglucomutase. The activity of hexokinase and aldolase was higher in the conducting bundles than in the surrounding petiolar parenchyma. Hexokinase activity was highest in August, the time of intense sucrose accumulation in the roots. A comparison of Kursanov's results with the earlier work done by Wanner (120) is of interest. Wanner found phosphoglucomutase and a number of phosphatases in the sieve-tube exudate of *Robinia pseudo-acacia,* but no other glycolytic enzymes. The paucity of enzymes in this case is caused by the nature of the exudate. As discussed before, there is strong evidence that this exudate is the translocated content of sieve tubes, contaminated with traces of torn-out and washed-out material. Although this contamination is small in the case of trees, and insignificant for osmotic consideration, it may show up quite clearly in enzymatic studies. The enzymes found by Wanner are either ordinarily stationary but torn out during the initial flush of exudation, or they may, less probably, represent actually moving material. In neither case would they have to represent a complete picture of the sieve-tube metabolism. Kursanov's results, on the other hand were obtained with whole conducting bundles which contain many cells other than sieve elements.

Evidence for a metabolic maintenance of sieve-tube semipermeability has been presented by Zimmermann (137). Sieve tubes of trees are highly turgescent even far from the leaves and several days after defoliation. It has been mentioned before that secretion into the sieve tubes is probably a metabolic process. Removal of sugars from the sieve tubes also appears to occur metabolically. Evidence for the removal of substances from the sieve tubes of white ash has been obtained by quantitative analysis of exudate following complete defoliation. The rapid drop in concentration of all the substances during the first few hours after defoliation reflects the natural rate of removal from the sieve tubes, ordinarily (in an undefoliated tree) apparent as a longitudinal concentration gradient. Conversion of stachyose into sucrose

after defoliation indicates α-D-galactosidase activity. This enzyme appears to be located on the sieve-tube cytoplasm in contact with the vacuole, removing galactose units from oligosaccharides and transferring them out of the vacuole. Neither galactose residues nor the enzyme appear in the exudate.

Removal of sucrose and mannitol, in contrast to the removal of galactose units which proceeds almost to completion, comes to a halt a few days after defoliation. These two substances then begin to accumulate. It seems that in the presence of leaves, sucrose and mannitol leave the phloem, while in the absence of leaves their re-entry is stimulated (137).

Another hormone-like effect produced by the leaves upon phloem translocation was reported by De Stigter (25). When *Cucurbita ficifolia* (designated F below) was grafted onto stocks of *Cucumis melo* (designated M below) (F/M grafts), perfect unions and normal growth was obtained. M/F grafts, however, were incompatible unless stock leaves remained on F. This incompatibility was found to be based upon phloem failure. Defoliation of the stock in M/F grafts resulted in rapid and specific collapse of the sieve tubes of the stock before any other incompatibility symptoms became visible. The number of stock leaves in M/F grafts was important. Growth was slow with a single stock leaf and starch accumulated in the top of the plant. With an increasing number of stock leaves, growth improved and the percentage of dry matter in the top decreased. In contrast to M/F, F/M/F grafts were successful after removal of the stock leaves. It seems that some specific factor from the leaves is needed for the proper functioning of the phloem.

Quantitative Aspects of Phloem Translocation

Concentration gradients.—Concentrations of sieve-tube exudates of trees are usually 0.4 to 0.6 molar. In an early paper, Pfeiffer (87) reported osmotic pressure gradients of 0.11 to 1.04 atm. per meter (various tree species, cryoscopic measurements). Huber *et al.* (56, 57) found concentration gradients about 0.012 mole per meter in red oak (refractometric measurements), and Zimmermann (135 to 137) about 0.01 mole per meter in white ash (paper chromatography). All of these gradients were positive (decreasing concentration) in the downward direction of the trunk during the summer months. Zimmermann (137) performed defoliation experiments with white ash, measuring the molar concentration gradient along the trunk before and a few hours after all the leaves had been stripped from the twigs. After defoliation, some of the individual gradients became negative and others remained positive, the total molar gradient thus disappearing completely.

If concentration gradients are of the above reported magnitude in all plants, then they would be very difficult to detect in small plants. This is reflected in an earlier attempt by Tingley (117) whose results show mainly dilution and special "sink" effects. Moreover, toward rapidly growing tissues (fruits, growing tips, etc.) the turgor gradient does not have to be followed

by a concentration gradient. This has been discussed by Münch (79) and Crafts (18).

Distribution patterns of radioactivity represent the total amount of tracer inside and outside the translocation channels. This is clearly indicated in the paper written by Vernon & Aronoff (118) who found label in both the starch and cellulose fractions of the stem of a bean seedling after 20 min. of $C^{14}O_2$ photosynthesis, even before any tracer had reached the roots.

Horwitz (54) has analyzed mathematically a number of models of short-term translocation patterns and compared these models with experimentally found distribution patterns of radioactive isotopes. Radioactivity decreases logarithmically along the stem from the place of application (6, 107, 114, 118). This agrees well with Horwitz's models for *en masse* and cyclosis-diffusion flow with irreversible loss of translocate to the surrounding tissue at a rate proportional to its concentration.

Translocation velocities.—Earlier reviews have reported that translocation minimum velocities in the phloem are of the order of magnitude of 10 to 100 cm. per hour. In general, this has been confirmed by recent papers, with occasional reports of somewhat higher values [up to 300 cm. per hour in young soybean seedlings (81)]. Nelson *et al.* (83) reported xylem velocities of 3000 cm. per hour in $C^{14}O_2$ photosynthesis experiments of 30 sec. duration. These experiments seem to show that even in photosynthesis experiments there may be a xylem component of the outflowing label.

Of particular interest are velocity measurements of simultaneously applied substances. Analyzing Swanson & Whitney's (114) data on the simultaneous movement of Cs^{137}, P^{32}, and K^{42}, Horwitz (54) indicates that differences in slopes of isotope distribution (Cs and P) need not indicate differences in translocation velocities, but may be attributable to a different rate of loss from the sieve tubes. Nevertheless, although the slopes of P and K were the same the rate of increase of the logarithms of their radioactivity with respect to time were different. Compared with the mathematical model, either the velocity or the cross sectional conducting area, or both, must be different for P and K. One may, perhaps, avoid this conclusion by assuming variable uptake and/or removal rates of one or both substances. Biddulph & Cory (6) studied the simultaneous movement of THO, P^{32}, and C^{14}. The plants were analyzed after 15, 20, and 30 min. C^{14} was recovered largely as sucrose, and P^{32} as phosphate or fructose-1,6-diphosphate (containing no C^{14}). Velocities calculated from data that include uptake into the sieve tubes were 87 cm. per hour for P^{32} and THO, and 107 cm. per hour for C^{14}. Losses from the phloem to the xylem were approximately 24 per cent for P^{32} and C^{14}, and 31 per cent for THO. In all cases the front of the THO stream showed a relatively large amount of tracer, undoubtedly caused by the readjustment of the hydrostatic situation in the water conducting system (xylem and cell walls) upon external application of water. The ratio THO/H_2O available to the phloem is probably greater during the initial THO surge than after the new equilibrium has been reached.

Volume of translocated material.—Early growth rate calculations by Dixon *et al.* (27), Münch (79), and Crafts (16, 21) have indicated a translocation rate of 20 to 100 cm. per hour if (*a*) the whole sieve-tube lumen is conducting and (*b*) sugars move as a solution of 10 to 28 per cent w/v concentration. Subsequently, these translocation rates have been shown to be of the right order of magnitude, and exudate concentrations are, indeed, between 10 and 28 per cent. Evidence is accumulating that movement is *en masse* in the form of a solution. Biddulph & Cory (6) found water (THO) to move together with phosphate-P^{32} and sucrose-C^{14} in the phloem. The argument that exudation only occurs in a few plants has lost its validity since aphids can be made to find the sieve tubes for us and delicately puncture them. Unfortunately, with Hartig's method the sieve-tube exudate of most plants, even deciduous trees, gets easily lost in the bark tissue. The rate of flow from aphid stylets was found to be 0.5 to 3 $mm.^3$ per hour in willow (53, 75, 122), 0.5 $mm.^3$ per hour in *Heracleum mantegazzianum,* and 0.05 $mm.^3$ per hour in *Picea abies* (131). Mittler (75) calculated the rate of flow in willow to correspond to the volume of 100,000 sieve elements per hour or a linear translocation rate of 500 cm. per hour. Weatherley *et al.* obtained somewhat lower values (100 cm. per hour).

Hill (53) and Weatherley *et al.* (122) obtained the full exudation rate from aphid stylets on willow even with short pieces of stem and irrigated strips of bark. They found that a minimal length of about 16 cm. of bark (800 to 1000 sieve elements) was necessary to give the full rate of flow. Continuous exudation from such pieces of bark indicates a rapid sealing of the cut ends of the sieve tubes and a switch-over in source of supply from leaves to storage cells [cf. the findings of Zimmermann (137) above]. When the xylem of the stem was perfused with sucrose or mannitol, the exudation rate declined and the exudate concentration rose.

On the Mechanism of Phloem Transport

This problem has been, and still is, the subject of appreciable controversy. It is beyond the purpose of this paper to discuss all the theories put forth over the past years. The reader will find such treatments in earlier reviews (18, 33). Discussion here will be restricted to publications subsequent to the latest review (33) and to what appears to the writer as being the two most plausible explanations of the phenomenon of long-distance transport in the phloem.

One of these views is presented by the mass-flow theory advanced by Münch (79). In its original form it included the whole symplast of the plant (all living cells). Today its functioning is thought to be restricted to the sieve tubes. Crafts (19) has outlined it briefly as follows:

> The phloem is visualized as an extended and ramifying osmotic system in which exists, because of metabolic processes, a gradient, or gradients, of concentration of osmotically active substances (assimilates). Because the phloem system has a com-

mon source of water in the xylem, absorption of water in regions of high concentration where synthesis is taking place brings about increased hydrostatic pressure, whereas utilization of assimilates in growth, storage, and respiration results in lowered concentration and hence lowered hydrostatic pressure where these activities are going on. Because of the gradients of hydrostatic pressure so created, solution moves from regions of synthesis to regions of utilization through the sieve tubes of the phloem.

Zimmermann (135 to 137) placed much emphasis on the metabolic aspect of such a mechanism. Sieve-tube semipermeability is visualized as a reversible enzymatic process of solute entry ⇄ exit (release into the sieve-tube lumen and uptake from there), the direction of this process possibly being controlled by a leaf stimulus (137). Such a mechanism would automatically, and at any time, direct the transport from source to sink. It has been pointed out earlier that entry into sieve tubes involves a number of steps. The same is undoubtedly true for the removal of substances. For example, there are many steps from the removal of a sugar molecule from the sieve-tube lumen to its final incorporation into a cellulose chain of a cambial wall. An interesting model of such a secretion process is represented by nectaries. The tissue associated with "pumping" is the secretory tissue, located between the supplying ends of the phloem and the epidermis, and composed of cells which differ from the surrounding parenchyma by their meristematic appearance (39). Secretion itself is a highly metabolic process, involving sugar transformation (42) and depending on the auxin level (73). In the companion cells of the phloem we would have an anatomically comparable "secretory tissue" for the loading and unloading of the sieve tubes (the last step of loading and the first step of unloading being located in the sieve-tube cytoplasm). With such "pumps" along the whole channel the above described mechanism could easily function; and the occasional absence of an ontogenetically associated companion cell (14) would have little effect upon the system. (The word "pump" may be somewhat misleading. If the release into the sieve-tube lumen and the absorption on the cytoplasm are enzymatic steps, then sugars would be removed from the sieve-tube lumen irreversibly as well as reversibly). In 1949 Kursanov & Zaprometov (69) suggested that phloem translocation takes place along an "absorption gradient" on the cytoplasm. This resembles the above described mechanism although it was thought to function directly.

The literature is full of apparent evidence against mass flow. Most of this is attributable to a lack of distinction between uptake and translocation and to a disregard of movement in the xylem. These, and other aspects such as bidirectional movement, have been discussed earlier. Translocation velocities are of the same order of magnitude for a wide variety of substances. That apparent velocities may vary slightly, depending on uptake and removal rates of the individual substances, has also been discussed. Even translocation in wilting plants can be explained, either through "suction flow" (40),

or by considering that secretion into the sieve tubes occurs against a concentration gradient. The sieve tubes may still be turgescent even though the surrounding tissue is wilting [cf. (18) exudation from wilting plants].

The requirements for osmotic mass flow are essentially fulfilled, at least in some deciduous trees, since the experimentally observed gradients are in the right direction and disappear upon defoliation. The side-wall cytoplasm of the sieve tubes must be regarded as semipermeable because of the long-retained turgescence of the sieve tubes after defoliation. Longitudinally the sieve tubes appear to be permeable because of ready exudation, over periods of days, from both aphid proboscides and from punctures left by withdrawn proboscides. In spite of this very solid evidence in its support, the osmotic mass flow theory has its weak point: the anatomy of the sieve plates. The reader will find a discussion of sieve-element anatomy in the recent review by Esau *et al.* (33).

From a physiological viewpoint we can only remind the reader of the finding of Crafts and others that sieve plates are immediately plugged upon severance. This is very evident when certain trees are tapped by Hartig's method. Exudation occurs only during a fraction of a second. Species like *Fraxinus americana,* in which exudation continues for hours or days, are the exception. Exudation from aphid stylets, by contrast, is continuous. The puncture, in this case, seems to be such a delicate one that plugging of sieve plates does not occur. Anatomical investigations on the basis of such observations may perhaps help to clarify this point. Unfortunately, we cannot look at sieve plates of intact, conducting sieve elements.

After having examined electro-osmosis and streaming potentials in xylem and phloem, Fensom (36) concluded that bio-electric forces are sufficiently large in magnitude to cause transport and that they operate in a direction to assist it. According to Fensom, sieve plates may become charged by respiration of sugars on one side, production of H^+ and HCO_3^-, and faster diffusion of H^+ through the barrier. Spanner (109), in a similar theory, suggests that electrical potentials are maintained across sieve plates by a circulation of potassium or some other ions in the direction of transport through the sieve plate and back outside the sieve elements. The circulation is thought to be caused by a divergence existing between the intensities of the active ion uptake on the two sides of the plate. The result would be a mass flow of solution through sieve tubes along a sawtooth pressure gradient.

Calculations with Poiseulle's equations show that if the sieve pores were open, the observed osmotic gradients in trees would be fully sufficient to cause a flow at the observed rates of translocation. If the pores are filled with stationary material, however, the required gradients would be far too high. The conditions would then be more favorable for electro-osmotic flow. The two mechanisms could easily operate together. While, in Münch's theory, flow is thought to be caused by secretion processes into and out of the sieve tubes, in Spanner's electrokinetic theory flow is triggered by secretion. The

use of Poiseulle's equation in the sieve pores is, of course, a rough extrapolation.

The weakest point of the osmotic mass flow theory is the unknown permeability of the sieve plates under undisturbed conditions. The electrokinetic theory, on the other hand, is still in its early speculative phase and needs much experimental support. The present value of such hypotheses perhaps lies less in their ability to explain a natural phenomenon in a more or less plausible way, than in their stimulating effect upon the search for more knowledge.

LITERATURE CITED

1. Aronoff, S., *Plant Physiol.*, **30**, 184–85 (1955)
2. Barrier, G. E., and Loomis, W. E., *Plant Physiol.*, **32**, 225–31 (1957)
3. Beer, M., *Proc. Intern. Botan. Congr., 9th congr., Montreal, Canada*, **2**, 26 (1959)
4. Belikov, I. F., *Doklady Akad. Nauk S.S.S.R.*, **120**, 904–6 (1958)
5. Biddulph, O., Biddulph, S., Cory, R., and Koontz, H., *Plant Physiol.*, **33**, 293–300 (1958)
6. Biddulph, O., and Cory, R., *Plant Physiol.*, **32**, 608–19 (1957)
7. Biddulph, O., Cory, R., and Biddulph, S., *Plant Physiol.*, **31**, 28–33 (1956)
8. Bollard, E. G., in *The Physiology of Forest Trees*, 83–93 (Thimann, K. V., Ed., Ronald Press, New York, N.Y. 1958)
9. Braun, H. J., *Ber. deut. botan. Ges.*, **70**, 305–22 (1957)
10. Bukovac, M. J., and Wittwer, S. H., *Plant Physiol.*, **32**, 428–35 (1957)
11. Bukovac, M. J., Wittwer, S. H., and Tukey, H. B., *Plant Physiol.*, **31**, 254–55 (1956)
12. Caldwell, J., *New Phytologist*, **29**, 27–43 (1930)
13. Chailakhyan, M.Kh., and Butenko, R. G., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **4**, 450–62 (1957)
14. Cheadle, V. I., and Esau, K., *Univ. Calif. (Berkeley) Publs. Botany*, **29**, 397–510 (1958)
15. Chen, S. L., *Am. J. Botany*, **38**, 203–11 (1951)
16. Crafts, A. S., *Plant Physiol.*, **8**, 81–104 (1933)
17. Crafts, A. S., *Am. J. Botany*, **26**, 172–77 (1939)
18. Crafts, A. S., *Botan. Rev.*, **17**, 203–84 (1951)
19. Crafts, A. S., *Hilgardia*, **26**, 287–334 (1956)
20. Crafts, A. S., *Hilgardia*, **26**, 335–65 (1956)
21. Crafts, A. S., and Lorenz, O. A., *Plant Physiol.*, **19**, 131–38 (1944)
22. Crafts, A. S., and Yamaguchi, S., *Hilgardia*, **27**, 421–54 (1958)
23. Curtis, O. F., *Am. J. Botany*, **16**, 154–68 (1929)
24. Curtis, O. F., and Asai, G. N., *Am. J. Botany*, **26**, 16s–17s (1939)
25. De Stigter, H. C. M., *Mededel. Landbouwhogeschool Wageningen*, **56**, 1–51 (1956)
26. Dickson, A. G., and Samuels, E. W., *J. Arnold Arboretum (Harvard Univ.)* **37**, 307–13 (1956)
27. Dixon, H. H., and Ball, N. G., *Nature*, **109**, 236 (1922)
28. Drawert, H., *Ber. deut. botan. Ges.*, **70**, 401–8 (1957)

29. Dugger, W. M., Jr., Humphreys, T. E., and Calhoun, B., *Plant Physiol.*, **32**, 364–70 (1957)
30. Erickson, L. C., *Science*, **125**, 994 (1957)
31. Esau, K., and Cheadle, V. I., *Proc. Natl. Acad. Sci. U.S.*, **44**, 546–53 (1958)
32. Esau, K., and Cheadle, V. I., *Proc. Natl. Acad. Sci. U.S.*, **45**, 156–62 (1959)
33. Esau, K., Currier, H. B., and Cheadle, V. I., *Ann Rev. Plant Physiol.*, **8**, 349–74 (1957)
34. Eschrich, W., *Protoplasma*, **47**, 487–530 (1956)
35. Eschrich, W., *Planta*, **48**, 578–86 (1957)
36. Fensom, D. S., *Can. J. Botany*, **35**, 573–82 (1957)
37. Fischer, A., *Ber. math.-phys. Kl. kgl. sächs. Ges. d. Wiss.*, **38**, 291–336 (1886)
38. Fischer, H., in *Encyclopedia of Plant Physiology*, **4**, 289–306; **6**, 924–34, 952–77; **8**, 610–36 (Ruhland, W., Ed., Springer Verlag, Berlin, Germany, 1958)
39. Frei, E., *Ber. schweiz. botan. Ges.*, **65**, 60–114 (1955)
40. Frey-Wyssling, A., *Jahrb. wiss. Botan.*, **77**, 560–626 (1932)
41. Frey-Wyssling, A., and Müller, H. R., *J. Ultrastructure Research*, **1**, 38–48 (1957)
42. Frey-Wyssling, A., Zimmermann, M., and Maurizio, A., *Experientia*, **10**, 491–92 (1954)
43. Gauch, H. G., and Dugger, W. M., Jr., *Plant Physiol.*, **28**, 457–66 (1953)
44. Gauch, H. G., and Dugger, W. M., Jr., *Maryland Agr. Exp. Sta. Tech Bull. No. A-80* (1954)
45. Gustafson, F. G., *Am. J. Botany*, **43**, 157–60 (1956)
46. Hartig, T., *Jahresberichte über die Fortschritte der Forstwissenschaften und forstlichen Naturkunde*, **1**, 125–68 (1837)
47. Hartig, T., *Allg. Forst. und Jagdztg.*, **36**, 257–61 (1860)
48. Hartig, T., *Lehrbuch für Förster* (J. G. Cotta, Stuttgart, Germany, 11th ed. 1877)
49. Hay, J. R., *Weeds*, **4**, 218–26 (1956)
50. Hay, J. R., *Weeds*, **4**, 349–56 (1956)
51. Hay, J. R., and Thimann, K. V., *Plant Physiol.*, **31**, 382–87 (1956)
52. Hay, J. R., and Thimann, K. V., *Plant Physiol.*, **31**, 446–51 (1956)
53. Hill, G. P., *A Technique for the Study of the Physiology of the Sieve Tubes* (M.S. thesis, Univ. of Nottingham, Nottingham England, 1955)
54. Horwitz, L., *Plant Physiol.*, **33**, 81–93 (1958)
55. Huber, B., *Ber. deut. botan. Ges.*, **66**, 341–46 (1953)
56. Huber, B., in *The Physiology of Forest Trees*, 367–79 (Thimann, K. V., Ed., Ronald Press, New York, N.Y., 1958)
57. Huber, B., Schmidt, E., and Jahnel, H., *Tharandt. forstl. Jahrb.*, **88**, 1017–49 (1937)
58. Kendall, W. A., *Plant Physiol.*, **30**, 347–50 (1955)
59. Kessler, G., *Ber. schweiz. botan. Ges.*, **68**, 5–43 (1958)
60. Koontz, H., and Biddulph, O., *Plant Physiol.*, **32**, 463–70 (1957)
61. Kulaeva, O. N., Silina, E. I., and Kursanov, A. L., *Fiziol. Rastenii Akad. Nauk, S.S.S.R.*, **4**, 520–8 (1957)
62. Kursanov, A. L., *Ann. Rev. Plant Physiol.*, **7**, 401–36 (1956)
63. Kursanov, A. L., *Izvest. Akad. Nauk S.S.S.R., Ser. Biol.*, No. 6, 689–705 (1957)
64. Kursanov, A. L., in *Radioisotopes in Scientific Research*, 4; *Proc. UNESCO*

Intern. Conf., Paris, 1957, 1st Conf. (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
65. Kursanov, A. L., Chailakhyan, M. Kh., Pavlinova, O. A., Turkina, M. V., and Brovchenko, M. I., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **5**, 3–15 (1958)
66. Kursanov, A. L., and Kulayeva, O. N., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 322–31 (1957)
67. Kursanov, A. L., Pavlinova, O. A., and Afanasieva, T. P., *Arch. Biochem. Biophys.*, **83**, 239–47 (1959)
68. Kursanov, A. L., and Viskrebentzeva, E. I., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **1**, 154–163 (1954)
69. Kursanov, A. L., and Zaprometov, M. N., *Doklady Akad. Nauk S.S.S.R.*, **69**, 89–92 (1949)
70. Leonard, O. A., *Hilgardia*, **28**, 115–60 (1958)
71. Leonard, O. A., and Crafts, A. S., *Hilgardia*, **26**, 366–415 (1956)
72. Mason, T. G., and Phillis, E., *Ann. Botany* (*London*), **50**, 455–99 (1936)
73. Matile, Ph., *Ber. schweiz. botan. Ges.*, **66**, 237–66 (1956)
74. Mittler, T. E., *Nature*, **172**, 207 (1953)
75. Mittler, T. E., *J. Exptl. Biol.*, **34**, 334–41 (1957)
76. Mittler, T. E., *J. Exptl. Biol.*, **35**, 74–84 (1958)
77. Mittler, T. E., in *The Physiology of Forest Trees*, 401–5 (Thimann, K. V., Ed., Ronald Press, New York, N.Y., 1958)
78. Mothes, K., and Engelbrecht, L., *Flora*, **145**, 132–45 (1957)
79. Münch, E., *Die Stoffbewegungen in der Pflanze* (Gustav Fischer, Jena, Germany, 234 pp., 1930)
80. Nelson, C. D., and Gorham, P. R., *Can. J. Botany*, **35**, 339–47 (1957)
81. Nelson, C. D., and Gorham, P. R., *Can. J. Botany*, **35**, 703–13 (1957)
82. Nelson, C. D., and Gorham, P. R., *Can. J. Botany*, **37**, 431–47 (1959)
83. Nelson, C. D., Perkins, H. J., and Gorham, P. R., *Can. J. Biochem. and Physiol.*, **36**, 1277–79 (1958)
84. Novitsky, Yu. I., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 243–53 (1957)
85. Pallas, J. E., and Crafts, A. S., *Science*, **125**, 192–93 (1957)
86. Pennell, G. A., and Weatherley, P. E., *New Phytologist*, **57**, 326–39 (1958)
87. Pfeiffer, M., *Flora*, **132**, 1–47 (1937)
88. Pollard, J. K., and Sproston, Th., *Plant Physiol.*, **29**, 360–64 (1954)
89. Pristupa, N. A., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **6**, 30–5 (1959)
90. Pristupa, N. A., and Kursanov, A. L., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 417–24 (1957)
91. Prokofyev, A. A., and Sobolev, A. M., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 12–20 (1957)
92. Prokofyev, A. A., Zhdanova, L. P., and Sobolev, A. M., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 425–31 (1957)
93. Putman, E. W., and Hassid, W. Z., *J. Biol. Chem.*, **207**, 885–902 (1954)
94. Resch, A., *Planta*, **52**, 121–43 (1958)
95. Resch, A., *Planta*, **52**, 467–515 (1959)
96. Reuter, G., and Wolffgang, H., *Flora*, **142**, 146–55 (1954)
97. Rice, E. L., and Rohrbaugh, L. M., *Plant Physiol.*, **33**, 300–3 (1958)
98. Röckl, B., *Planta*, **36**, 530–50 (1949)
99. Rohrbaugh, L. M., and Rice, E. L., *Plant Physiol.*, **31**, 196–99 (1956)

100. Rouschal, E., *Flora,* **135,** 135–200 (1941)
101. Sax, K., *J. Arnold Arboretum (Harvard Univ.),* **35,** 251–58 (1954)
102. Schnepf, E., *Planta,* **52,** 644–708 (1959)
103. Schumacher, W., *Jahrb. wiss. Botan.,* **73,** 770–823 (1930)
104. Schumacher, W., and Kollmann, R., *Ber. deut. botan. Ges.,* **72,** 176–79 (1959)
105. Schumacher, W., and Lambertz, P., *Planta,* **47,** 47–52 (1956)
106. Sievers, A., *Flora,* **147,** 263–316 (1959)
107. Sisler, E. C., Dugger, W. M., Jr., and Gauch, H. G., *Plant Physiol.,* **31,** 11–17 (1956)
108. Skok, J., *Plant Physiol.,* **32,** 648–58 (1957)
109. Spanner, D. C., *J. Exptl. Botany,* **9,** 332–42 (1958)
110. Strugger, S., *Arbeitsgemeinschaft für Forschung des Landes Nordrhein-Westfahlen,* Heft 21a, 33–54
111. Strugger, S., and Butterfass, Th., *Planta,* **45,** 549–56 (1955)
112. Swanson, C. A., in *Atomic Energy and Agriculture,* 123–38 (Am. Assoc. Advanc. Sci., Washington, D.C., 1957)
113. Swanson, C. A., and El-Shishiny, E. D. H., *Plant Physiol.,* **33,** 33–37 (1958)
114. Swanson, C. A., and Whitney, J. B., Jr., *Am. J. Botany,* **40,** 816–23 (1953)
115. Teubner, F. G., Wittwer, S. H., Long, W. G., and Tukey, H. B., *Michigan Agr. Expt. Sta. Quart. Bull.,* **39,** 398–415 (1957)
116. Thorne, G. N., *Ann. Botany (London),* **22,** 381–98 (1958)
117. Tingley, M. A., *Am. J. Botany,* **31,** 30–38 (1944)
118. Vernon, L. P., and Aronoff, S., *Arch. Biochem. Biophys.,* **36,** 383–98 (1952)
119. Wanner, H., *Ber. schweiz. botan. Ges.,* **63,** 162–8 (1953)
120. Wanner, H., *Ber. schweiz. botan. Ges.,* **63,** 201–12 (1953)
121. Wartenberg, A., *Ber. deut. botan. Ges.,* **70,** 263–74 (1957); **71,** 169–76 (1958)
122. Weatherley, P. E., Peel, A. J., and Hill, G. P., *J. Exptl. Botany,* **10,** 1–16 (1959)
123. Wedding, R. T., and Riehl, L. A., *Am. J. Botany,* **45,** 138–42 (1958)
124. Willenbrink, J., *Planta,* **48,** 269–342 (1957)
125. Wolffgang, H., and Mothes, K., *Naturwissenschaften,* **40,** 606 (1953)
126. Woolley, J. T., Broyer, T. C., and Johnson, G. V., *Plant Physiol.,* **33,** 1–7 (1958)
127. Yamaguchi, S., and Crafts, A. S., *Hilgardia,* **28,** 161–91 (1958)
128. Ziegler, H., *Planta,* **47,** 447–500 (1956)
129. Ziegler, H., *Flora,* **144,** 229–50 (1957)
130. Ziegler, H., *Planta,* **51,** 186–200 (1958)
131. Ziegler, H., and Mittler, T. E., *Z. Naturforsch.,* **14b,** 278–81 (1959)
132. Zimmerman, P. W., and Connard, M. H., *Contribs. Boyce Thompson Inst.,* **6,** 297–302 (1934)
133. Zimmermann, M. H., *Ber. schweiz. botan. Ges.,* **63,** 402–29 (1953)
134. Zimmermann, M. H., *Plant Physiol.,* **32,** 288–91 (1957)
135. Zimmermann, M. H., *Plant Physiol.,* **32,** 399–404 (1957)
136. Zimmermann, M. H., in *The Physiology of Forest Trees,* 381–400 (Thimann, K. V., Ed., Ronald Press, New York, N.Y., 1958)
137. Zimmermann, M. H., *Plant Physiol.,* **33,** 213–17 (1958)

VERNALIZATION AND ITS RELATIONS TO DORMANCY[1]

By P. Chouard

Laboratory of Plant Physiology, the Sorbonne, University of Paris, Paris, France

HISTORY AND DEFINITION OF VERNALIZATION

In temperate countries, the seed of winter cereals must be planted before the end of winter in order to fruit within 12 months of sowing. By contrast, spring cereals, which generally can not survive the winter cold, flower soon after a spring sowing. The very notion of vernalization originated from this early agricultural experience.

Systematic research was undertaken on this subject as early as 1857, by Klippart (128). He showed that among the various climatic factors of winter, the determining factor is the cold temperature to which the young plants are subjected for a few weeks; this makes the winter cereals capable of flowering soon after the return of a warmer temperature. This notion was extended to other plants by von Seelhorst in 1898 (237) and much more extensively by Gassner in 1918 (89), who showed the great variety of "cold requirements" (measured by minimal chilling time) displayed by numerous species known as "biennials" or "winter annuals" as opposed to "spring plants" or "summer annuals" which have no significant chilling requirement for flowering. Gassner also showed that in winter cereals the early swollen germinating seed is already sensitive to the specific cold effect. Finally, in 1928, Lysenko (155) established that slight imbibition (e.g., 50 parts water to 100 parts dry matter) makes the cereal seed susceptible to this action of cold without inducing the excessive germination that could prevent use of a sowing machine. It was Lysenko who gave a name to the phenomenon. As spring cereals are called Jarovoe in Russian (from "Jar": formerly fire, or the god of spring), he called the process which makes a winter cereal behave like a spring cereal "Jarovization," and translated the word in his own English, French, and German translations into "vernalization" (Latin *vernum* meaning spring). Some French authors use the word "printanisation" but vernalization is most commonly used. In the next 20 years, a tremendous number of studies were undertaken on vernalization, mostly by agronomists, to quantitize the data on the process in cereals and in numerous other cultivated plants, in order to facilitate growing a crop following late sowing.

From their study of vernalization, Lysenko and his followers built their famous phasic theory of the stadial development of plants (156, 157) and

[1] The survey of literature pertaining to this review was concluded September 1959.

felt they had confirmed Mitchourin's theory of the transmissibility of certain characters acquired by adaptation. Two main groups have studied the physiology of vernalization on an accurate experimental basis: Gregory and Purvis (97 to 105, 206 to 215) in London using the winter rye Petkus; and Melchers, Lang *et al.* (139 to 146, 168 to 178) at Tübingen, with the biennial strain of henbane (*Hyoscyamus niger*). During all the development of that literature, "vernalization" was used with various meanings. Such disorder has to be clarified to prevent never-ending confusion. The original restricted meaning was: the substitution of chilling of a plant for the natural exposure to winter in order to make possible the initiation of flower primordia later. Vernalization induces or hastens the development of the capacity for flowering and although its action is not visible at first, it appears as an aftereffect. Chilling is the normal agent.

The derived meaning is: any physiological effect of chilling corresponding to the awakening of nature in spring. In this context any effect of chilling that has a forcing effect is called vernalization. A further derived meaning is: any physiological action stimulating the capacity for flowering, whatever the agent. In this sense vernalization was obtained by heat (e.g., 205) or by cold, by long days or short days, by light or dark, by nutrition, etc.

In this review, the original restricted meaning is used because it is the most precise and the nearest to the initial idea; this is implicitly admitted in the works of Gregory, Purvis, Melchers, and Lang, and is explicitly used by Wellensiek (274).

We reject the first derived meaning for it is expressed by the "breaking of dormancy." During dormancy, all organs or their primordia pre-exist, but either do not grow or grow slowly. The breaking of dormancy (often induced by chilling) removes growth inhibitions and allows an active growth as soon as favourable conditions return but does not directly cause the formation of new kinds of organs as does vernalization. The breaking of dormancy belongs to the physiology of growth or of vegetative development; vernalization belongs to the physiology of reproductive development. As an example, chilling potatoes to increase tuber yield, though commonly but wrongly named "vernalization," merely utilizes an aftereffect of cold on vegetative vigour and has nothing to do with the specific aim of vernalization which is to induce the capacity for flowering. Inducing this capacity in a non-dormant rosette of biennal black henbane is true vernalization. Inducing dormant flower buds of lilac to grow and expand is breaking dormancy—not vernalization. Chilling is used in both of the examples, and relations between these two distinct but related phenomena will be discussed later.

I also reject the second derived meaning for it covers very different physiological phenomena such as photoperiodism, nutrition, or normal acceleration of growth and ageing by temperature rise. These phenomena are not related to the primary process of vernalization; i.e., a transformation of a winter plant into a spring plant. For instance, I shall not discuss here the so-called "heat-vernalization" which, in practice, has referred to such vari-

ous notions as normal growth acceleration by moderate heating, the phenomenon of thermoperiodicity, and sometimes experimental errors (241).

Another point to be emphasized concerning vernalization is that the process is revealed by an aftereffect. It is essentially a "preparatory process" to flowering, but is not the flower initiation process itself. In photoperiodism, the inductive photoperiod (or "euperiod") prepares for, as well as initiates, flower primordia. In bulbous Irises, subgenus *Xiphion*, studied by Blaauw (16), temperatures lower than 17° to 20°C. are necessary to initiate flower primordia, and this kind of chilling is not vernalization. During true vernalizing chilling, a sufficiently cold temperature is used, not to initiate flower primordia, but to create the capacity for subsequent flowering.

In summary, I will deal here with "vernalization" defined as follows: "The acquisition or acceleration of the ability to flower by a chilling treatment."

General reports or reviews on vernalization have been published during the last 20 years in Russian (157, 219), German (107, 139, 141, 172, 173, 177), French (36, 38, 41, 42, 63, 239, 274), Dutch (271, 277), and English (98, 140, 178, 183, 257, 279). In 1952 Lang published a general study on the physiology of flowering containing an excellent review of vernalization in the *Annual Review of Plant Physiology* (140). The story of vernalization of Petkus rye and henbane is so well described by Gregory (98) that no long description is necessary. The most commonly read book on this topic is probably *Vernalization and Photoperiodism* (183) published in 1948 by Murneek and Whyte; that fact explains why the present study deals mainly with investigations conducted between 1948 and 1959. Numerous important studies published before 1948 and here omitted appear in other references (140, 183, 279).

VARIOUS ASPECTS OF VERNALIZATION IN DIFFERENT SPECIES OF PLANTS

In order to work on a reliable basis I will first review the experimental data now available about vernalization in typical different species.

Vernalization of Cereal Grains with Special Reference to the Rye Petkus

Cereals are monocarpic plants, growing initially as short rosettes but with elongated stems, the culms, when flower primordia have been formed. Nevertheless, they must be studied separately from other ordinary rosette plants like henbane because they have the peculiar ability to be vernalized in the seed stage, and the very large numbers of researches dealing with these plants have been conducted by trials using grain vernalization. The classical researches of Gregory and Purvis upon the rye Petkus are well known, so that only a short résumé is necessary.

"Petkus," a cultivated variety of *Secale cereale* consists of two races. The spring race is a typical annual rosette plant. When planted during the

spring under long days, the primary axis produces seven leaf primordia and then forms the young ear, recognizable by double ridges on the sides of the apex. In short days, it produces about 25 leaves before earing and may be described as a quantitative long-day plant. The winter race is a typical biennial rosette plant. When planted during spring, under long-day conditions, the primary axis produces 25 leaf primordia before earing, and 16 under short-day conditions; but if the seed has been previously chilled, it grows exactly like the spring variety (205, 206). The vernalizing effect of chilling has been studied over long experimental periods in this plant. Ear primordia have been formed under long-day conditions after the seventh leaf, even sometimes after the sixth, when chilling has been extended up to 90 days (94). Thus, in both strains the first six or seven lateral primordia formed by the apex are pre-determined as leaves, and "ripeness to flowering" is reached at the sixth or seventh leaf. From the seventh to the twenty-fifth leaf level, primordia are "labile" and can either give leaves and tillers exclusively, according to the photoperiodic treatment in the spring race, or in the winter race, according to the degree of vernalization and the subsequent photoperiod in accordance with the above interactions. The "labile" character of the seventh to twenty-fifth primordia is evidenced by the pre-inflorescent structure observed in the apex during this period (204). It is to be noticed that short days can here replace part of vernalization (94, 161, 206, 207, 266) as if the plant was a "facultative short-day" plant in relation to flower initiation. This is true at least as long as the winter strain has not been chilled; many cereal species show the same behaviour but it has not been shown that this last point is universally true. In all winter cereals, however, after vernalization the plant is a quantitative long-day plant.

The effectiveness of the vernalizing treatment may be measured either as the inverse of the cold exposure necessary to obtain the same result, e.g., the level of complete vernalization defined by earing after the seventh leaf under long days; or by the reduced number of leaves formed before ear initiation (this reduction ranging from 25 to 7 leaves); or else by the "score" (206, 207, 210), a statistical evaluation of the results observed on earing and emerging.

The effectiveness of chilling depends on several conditions.

(*a*) There must be a sufficient imbibition of water not necessarily the maximum, but at least the minimum imbibition necessary to allow the vernalizing process while low enough to hinder seedling growth, according to Lysenko's and Tolmacev's method (156, 258). In cereals, the appropriate water content is about 45 to 50 per cent of the dry weight, which means that 30 parts of water are normally required for 100 parts of air-dried mature seeds, with some variations according to the species or variety (80, 84, 97, 99, 106, 152). The two methods, maximum or minimum imbibition, do not always give exactly the same results in vernalization (94).

(*b*) The vernalizing process cannot begin before a period of activation

(the time necessary to induce the first apparent respiratory exchanges in the recently imbibed grain) of about 10 to 24 hr. at 15 to 18°C. after the beginning of soaking.

(*c*) The presence of air (oxygen) is absolutely required.

(*d*) An appropriate duration and temperature of chilling are needed. The effective low temperature is between +1 and 6 to 7°C. with a widespread optimum. It decreases from 0 to −4°C. and disappears completely below −6°C. It also decreases above +7°C. and disappears completely between 12 and 14°C. (89, 107, 108, 210, 220, 222, 223). Above 15 to 17°C. the devernalizing effect of warm temperatures begins (214, 219).

In immature embryos, as in mature seeds or seedlings, the time required for complete vernalization is about the same: about 40 to 45 days in the winter strain of Petkus rye. A longer vernalizing chilling does not greatly modify the result; however, if it exceeds three or four months it seems to become less effective (210). When the treatment is applied to an older plant the minimal exposure to vernalizing chilling that leads effectively to the immediate acquiring of the flowering ability, decreases with age (81).

Respiration inhibitors and growth inhibitors inhibit the vernalizing action when they are applied at concentrations that completely stop respiration and growth. But low hydration or imbibition with a highly osmotic solution greatly slows down growth without reducing anything in the vernalizing process (155, 258). From this fact originated Lysenko's absolute distinction between growth and development (156, 279). By contrast, some other inhibitors applied at moderate rates can inhibit vernalization without stopping growth significantly (28, 48) and can be called "anti-vernalizing" agents.

The age of the plant in which chilling can effectively induce vernalization is extremely precocious. The immature embyro within the milky caryopse can be vernalized, either because of the cold nights occurring during late summer maturation in subarctic countries (131, 288), or local chilling of the ear on stalk (100).

Gentle drying of the wet seeds during chilling stops the vernalizing process but does not delete the acquired stage of vernalization. Interruptions of chilling by mild temperatures around 14 to 15°C. do not markedly alter the stage already reached in the vernalizing process. Alternating temperatures do not reduce the minimum number of required chilling days. Interruptions at 20°C. partly throw back the characters acquired but strengthen them when prolonged (86, 233). Under natural conditions the various cereal races have different chilling-time requirements that correspond to their adaptation to their native climate and to the fact that resistance to death by cold decreases during earing (219).

Devernalization instigated numerous discussions. The Russian authors observed that hot temperatures partly offset the vernalization effects (8, 77, 95, 255); but under the influence of the development of all plants through successive irreversible stages (7, 157 to 159), devernalization was

denied in various forms and nuances or interpreted as not being a real stage reversion (219, 248, 252). Purvis and Gregory have greatly enlightened the problem (101, 102, 214, 215). Two techniques ensure rapid devernalization: (*a*) exposure to about 20°C. in a damp atmosphere lacking oxygen (this exposure must not exceed about 1 to 5 days for it is most severe and would eventually kill the plants (102, 104); and (*b*) the exposure in ordinary damp atmosphere at about 35°C. for a longer period of about 8 to 12 days (101, 105, 213).

The effectiveness of both these treatments depends on the interval between the end of vernalizing chilling and the beginning of devernalization. When vernalization has been thoroughly achieved, a return to 15 to 20°C. fixes the vernalized state in a few days; at 4 to 5°C. the fixing requires several weeks. During the fixation process further devernalization loses its effectiveness (214). Revernalization may be achieved by another chilling.

The embryo itself directly perceives the chilling vernalizing effect (103, 207, 242), not the endosperm as was first thought (132, 165 to 167). Excised embryos and mere cauline fragments of the embryo, even when separated in the dry state before any diffusion of endosperm products, can be vernalized when imbibed (103, 113, 130, 190, 207). They must, however, be supplied with the sugars normally coming from endosperm. With their own metabolites, excised embryos can be but partly vernalized, even less when they have been starved in warm conditions without sugar before chilling. A sugar supply gives them back the complete ability of being vernalized (provided that starvation has not been excessive), but then a certain lag time will be observed which corresponds to the synthesis from sugar of some metabolites required for vernalization (99, 208 to 210). In winter cereals and some other perennials, minerals (3, 135, 185b) or vitamins (56) may enhance vernalizability, perhaps in relation to more active respiration. The meaning of the physico-chemical properties presented by vernalized meristems will be discussed later. The earlier emergence of the first leaves of cereals and most of all the smaller size of the first two leaves characterizes the vernalized state (108).

The vernalized state is not here transmitted by grafting, which is not generally successful in monocotyledons. But the vernalized state is communicated without reduction through cell divisions, i.e., in an autocatalytical way to all meristematic tissues originating at the apex of the embryo, e.g., to tillers even though they are formed after the vernalizing treatment (18, 108, 207).

In cereals the organs already formed (young leaves, young culms) are most responsive to the elongating effect of gibberellin, but this substance cannot cause them to flower and cannot therefore replace vernalization by chilling (154). On the contrary, soaking nonchilled winter-strain seeds in natural extracts from vernalized embryos of rye (215) or of vernalized peas (116, 117) seems to be able to replace part of the cold vernalizing effect.

But for slight quantitative differences, these fundamental rules of the vernalization of winter rye Petkus are the same for all cereals; especially for wheat (31, 82, 85, 147, 152, 161, 162, 229, 266, 282 to 284), barley (12, 70), oats (155 to 159, 202, 219, 239), rice (58, 244, 245) and different cereals (60, 89, 90, 155 to 159, 202, 219, 239, 252, 266).

Vernalization of Biennial Dicotyledons in Rosettes

The classical case of henbane.—Our classical information concerning vernalization was based on henbane as much as on Petkus rye, because of Melchers' and Lang's remarkable work (92, 139 to 146, 168 to 178, 228, 287). They worked together in Tübingen with other collaborators for a long time. In brief, I will only emphasize the differences observed between the behaviour of henbane and the cereals.

Here again the species, *Hyoscyamus niger* consists of two physiological races, the biennial and the annual strains. The important differences relative to the cereals follow below.

(*a*) Neither the seed nor the immature embryo have yet been vernalized; chilling is only effective on plants at least ten days old and in the rosette stage. The capacity for vernalization increases until the thirtieth day and then stays constant for a long time. That is to say, in the terms used by Wellensiek, this plant presents a "juvenile phase" for the first ten days of life after germination since the plant cannot feel the vernalizing effect of cold before the rosette is ten days old; or in my own terms, the plant reaches its "ripeness for vernalization" at that age.

(*b*) The leafy plant is strictly a long-day type.

(*c*) The winter strain has an obligate requirement for vernalization.

(*d*) Partial replacement of vernalization by previous exposure to short days is not possible.

(*e*) This plant shows a remarkable ability for grafting and for the transmission of vernalization from donor to receptor through grafts (168 to 172).

The effectiveness of vernalization can be estimated by the inverse of the number of days between the end of chilling and the beginning of shooting at 20 to 25°C. under long days. The optimum temperature for vernalization is lower and more widespread for the longer chilling periods. As an example of temperature and exposure period, it ranges between +3 and +6°C. for 42 days of treatment yielding complete vernalization and a short lag time before shooting of about ten days. Over-vernalization does not seem detrimental. Daily alternate temperatures have no increased effect (186b). During chilling, oxygen is indispensable and light is useful.

Henbane is also known as having a particular photoperiodic behaviour: its critical photoperiod ranges from 11.5 hr. at 20.5°C. to 8.5 hr. at 11.5°C. When plants are quite vigorous, with reserve food in the root, defoliation

is followed by flowering even under short days. Suppressing respiration by anaerobiosis in the dark, or sugar infiltration into leaves, also induces flowering under short days. In the biennial variety, vernalization is acquired by chilling the vegetative point and not by chilling the leaves or roots only. Acquired vernalization is maintained for a long time even after more than 100 days under short-day conditions at 20°C. It can be acquired by split chilling periods interrupted by mild temperatures.

As in cereals, devernalization can be obtained either by anaerobiosis or more easily by raising the temperature to 35°C. for a few days, provided that the devernalization treatment be applied immediately after the end of chilling. An interval of 3 to 4 days at 20°C. is sufficient to fix the vernalized state definitively. After devernalization, revernalization is also possible.

Graft transmission of the vernalized state is one of the most extraordinary properties displayed by henbane (169 to 171, 176 to 178). A donor scion can be grafted generally by side-grafting onto the rosette; the scion either is a branch or a separate leaf from one of the numerous Solanaceous species among which all possible types of vernalization or photoperiodic requirements are found. By this means, Melchers succeeded in making a nonvernalized, biennial, black henbane receptor flower by grafting with a donor. The donor could be a fragment from an individual of the same strain previously vernalized and exposed to long days, or a fragment of the annual strain, or a fragment from the *Hyoscyamus albus* species which is indifferent to vernalization and photoperiodism, or from annual species, or from varieties that are photoperiodically insensitive (such as *Petunia hybrida, Nicotiana silvestris*), or even *Nicotiana tabacum* var. Maryland Mammoth, the famous short-day annual. Since a leaf of this latter plant exposed to long days cannot bring its own buds to flower, it is not a donor of the so-called flowering hormone or "florigen"; however, because it induces flowering in nonvernalized black henbane, it must contain another sort of hormone which is still only speculative, called "vernaline" by Melchers (172). Zeevaart (289) confirmed these facts and demonstrated the same results using a potato branch as donor. The potato donor is more active when defoliated. Tomato, on the other hand, does not transmit the flowering ability under these same conditions. Except for the tobacco, Maryland Mammoth, the donor must always be floriferous itself. Reciprocally, a nonvernalized, long-day, black henbane cannot be used as an effective donor to make a leafless Maryland Mammoth branch flower. The black henbane does act as a donor if previously vernalized. The theoretical concept concerning vernalization have been based mostly on these remarkably successful experiments on the graft transmission of the vernalized state. But, as has just been seen, their success is not general and in receptors other than henbane, such a transmission is only quite rarely successful.

Neither the serological properties (106) nor the measurements of respiratory intensity (112) have shown any significant differences between nonvernalized and vernalized plants.

Lang first successfully substituted treatment with gibberellin for vernalizing chilling using the biennial black henbane (142, 143). Whereas the formation of flower primordia precedes shooting in vernalization by chilling, shooting begins first and flowering follows later when gibberellin is used (263).

Other biennial plants in rosettes.—Studies with other species show that the classical data based on henbane need modification.

The industrial importance of beets resulted in numerous studies some time ago (12, 24, 51, 182, 192, 250, 251). Sugar beets, as well as mangelwurzel and other analogues, generally have an absolute vernalization requirement and their flowering depends absolutely on long days. Industrial sugar production utilizes these properties: plants must be sown late enough to suffer less cold than would vernalize them yet early enough to enjoy the longest possible period for vegetable growth and sugar accumulation in the tap-root. A sowing date can be fixed according to the variety and climate. By contrast, intentional vernalization yields flowers and seeds within the very year of sowing. Stout (251) showed that grafting a vernalized stock on a nonvernalized stock transmitted the flowering ability to the latter; this was confirmed by Margara (164).

More recent studies (275) show that vernalization can be achieved in very young plants and even in seeds, but responsiveness increases with age, beginning with the second month. Chilling rosettes deprived of most of their tap-roots did not give a vernalizing effect even though chilling only the root has no effect at all; therefore, in order to respond to the vernalization treatment, the vegetative point must receive what the root supplies to it (135).

Margara (164) extended his investigations to the various wild and cultivated *Beta* species that display the greatest variety in their physiological requirements. While the flowering of sugar beets and analogues generally requires absolute long days, the induction of shooting after vernalization can also occur in short days.

Short days show a quick and strong devernalizing effect which can act long after the end of chilling and when vernalization is already expressed by shooting or even by early flowering. The return to short days brings back the formation of perched rosettes and none of these plants reflower or grow if they are placed under long days again; everything must be revernalized by chilling. However, this revernalization is particularly difficult in more or less devernalized perched axillary rosettes. Gibberellin induces shooting but not flowering without chilling even for those perched rosettes about one metre high. In spite of prolonged gibberellin treatment, shooting finally stops, perched rosettes form again and now seem resistant to prolonged vernalization treatment.

In *Beta maritima,* which requires less vernalizing chilling, the terminal bud is somehow protected against initiation of flowers and the flowering that follows vernalization occurs much like that described later for *Geum,* by the shooting of axillary buds while the main rosette ensures a maintenance of the perennial state. In this species, gibberellin entirely replaces cold in inducing flowering as well as shooting.

The small crucifer, *Arabidopsis thaliana,* in rosette presents the advantages of quick growth and small dimensions, and displays all degrees of vernalization requirements from none at all to absolute (136, 138, 290). Napp-Zinn's investigations (184 to 186b) recently made the variety "Stockholm" of this carefully selected species a classical research material. This variety absolutely requires vernalization to be able to flower, but it shows successive possible periods for vernalization, devernalization, and anti-vernalization.

Normally, the treatment consists in soaking very small seeds for 24 hrs. at 20°C. followed by 9 to 15 weeks from −3.5° to +4 to 5°C. A long previous soaking of seeds delays flowering (anti-vernalizing effect), with the optimum vernalization obtained by 10 weeks at 2°C. Heat devernalization can be completely effected immediately after chilling no matter what chilling period is applied to the seeds. Heat treatment after germination can also delay flower formation, whether or not vernalization occurs later. The vernalized state is fixed by a stabilizing treatment at 20°C. for seven days. Revernalization is possible. The number of leaves on the main stem is related to the time of flowering: heat treatment before or after vernalization or after stabilization reduces the number of leaves by about 10 to 20 leaves compared to that of untreated plants flowering at the same age.

Gibberellic acid applied to the seeds of races that have low or facultative chilling requirements replaces the action of cold if the treated plants are grown under long days. Indoleacetic acid has a similar but weaker effect on plants grown under natural autumn days after the treatment, and a still weaker effect under long-day conditions. This action may sometimes be additive to that effected by gibberellin, whereas triiodobenzoic acid is antagonistic.

In contrast, true "winter"varieties that have an obligate chilling requirement do not respond to gibberellin. Young rosettes of nonvernalized plants of these varieties that are more resistant than older ones to vernalization by chilling, are more responsive to gibberellic acid, which induces them to shoot and flower. Then, as these rosettes grow and become susceptible once again to the vernalizing effect of cold, they grow less susceptible to gibberellic acid. Moreover, contrary to the situation in henbane, when cold and gibberellin cause shooting and flowering they simultaneously determine the initiation of the inflorescence and of shooting (228).

Salad lettuce (*Latuca sativa*), depending on the variety, responds in different degrees to vernalization, which is not, however, an absolute

requirement for flowering (243, 267). They normally have a long vegetative growth period as a rosette, the leaves of which constitute the head. Under long warm days the axis shoots up, the head-leaves separate, and flowers appear. Head formation, followed by shooting, depends on light intensity, period, and colour, as well as the relative temperatures of day and night. The response to these factors, however, is modified by vernalization. The extent of this effect depends on the temperature to which the seeds (after soaking), or the seedlings, are exposed. Exposure of the Great Lakes variety for a minimum of 13 days, or better for 20 days, at about 3°C., followed by growth at a night temperature above 13°C. results in a rapid acceleration of shooting and flowering without head formation. At lower night temperatures a head is formed but flowering occurs sooner than in nonvernalized controls. Short interruptions of the cold treatment for a few hours per day with temperatures of 20° to 35°C. do not decrease the effectiveness of the vernalizing treatment (cf. mainly Rappaport and Wittwer (216, 217). Vernalized seeds release half as much indoleacetic acid as nonvernalized seeds when extracted with ethyl ether (87).

Other salad plants, like endive (*Cichorium endivia*), which respond quantitatively to vernalization and photoperiodism, are induced to bolt and flower by use of gibberellin (109).

Spinacia oleracea is considered to be a strictly annual, long-day plant with no vernalization requirement. Chilling the wet seeds of certain varieties however (e.g., Matador), between 0° and 5°C. for five days, causes plants to flower earlier under subsequent long days and to produce more flowers and seeds (126). The flowering of other varieties is generally accelerated by vernalization of wet seeds for two weeks at 3°C. (127, 187). Chilling treatments of 10 to 40 days speed up shooting, compared to the control, if shooting takes place under long 17-hour days preceeded by a short-day period of growth. Such acceleration is less marked, when compared to the control, if growth is entirely carried out under continuous days. Plants of the variety Nobel will flower under short days when the seeds have been vernalized (264).

Campanula medium was the object of a remarkable study by Wellensiek *et al.* (268, 270, 272). The seed is not vernalizable and rosettes maintained at a warm temperature under short or long days never flower. If such rosettes are exposed at the age of four months to cold temperature for at least four weeks and are then returned to a warm temperature combined with long days, they shoot and flower. If, upon the return to the warm temperature, they are subjected to short days, they remain in rosette. These results show this plant to be an absolute biennial that flowers only after vernalization followed by long days, similar to the biennial *Hyoscyamus niger*. *C. medium* differs from *H. niger* in that neither defoliation nor grafting can make it flower without previous chilling. On the contrary, if this plant is grown in rosette up to the age of three months in a warm temperature and

under short days, and then exposed to the warm temperature again but in combination with long days, it shoots and flowers. Here the early growth under short days replaces the vernalization by chilling. This property may be called "Wellensiek's phenomenon."

Gibberellin causes most *C. medium* plants to shoot under long days but after a certain number of repeated applications, growth stops when the plants are about 20 to 30 cm. and perched rosettes form with no flowering (273). I personally obtained contrary results with certain individuals after the same treatment (43). This is not in contradiction to Wellensiek for I probably had plants from unselected horticultural strains, whereas Wellensiek's plants were selected genetically from ancestors that gave a variety of reactions to chilling. Among my own plants, some not only show "Wellensiek's phenomenon," but are also unable to flower under long days without vernalization in spite of gibberellin applications. In such cases, some of my plants, after they have formed the perched rosette, resume shooting when given repeated gibberellin applications and then after 20 to 30 cm. of elongation, set in rosette again for a few weeks and so on—until they are several metres high. This is of considerable interest since it demonstrates the different responses given by separate individuals that differ by slight genetical characteristics not evident under ordinary cultivation.

Oenothera biennis and other *Oenothera* constitute one of the most complex genera in relation to vernalization. The species must be determined most carefully. Mrs. Picard and I have observed (35, 39, 42, 43, 201, 202) that *Oenothera biennis* (in the pure race obtained from the Munich botanical garden) has an intense and obligate requirement for chilling followed by long days if it is to flower. Up to now, natural and complex winter chilling proved much more effective than attempts at artificial chilling and alternating temperatures appeared more effective than continuous cold. Seed vernalization has been ineffective. After natural vernalization, the plants exposed to short days remain in rosette but soon show that they have lost their vernalized state, for they do not react any more to long-day exposures. After a long devernalizing period under short days, their old rosettes even seem particularly difficult if not impossible to revernalize. Gibberellic acid repeatedly applied to nonvernalized rosettes causes shooting to a height of 20 to 30 cm., under long days, followed by another perched rosette which then seems almost fully resistant to further treatments.

With *Oenothera lamarckiana* (from the forest of Fontainebleau), and *O. parviflora* (from Hooge Weluve near Arnhem), on the other hand, even though the chilling and long-day requirements are also qualitatively obligate, they are much easier to satisfy quantitatively. Artificial chilling in a cold room under low illumination for 12 hr. per day at 3 to 4°C. for 1 to 2 months generally succeeds. A more effective treatment appears to be with temperatures alternating every week or half week at 3° and 10 to

12°C. successively. Late devernalization by short days is also evident and revernalization is possible. Repeated gibberellin applications to nonvernalized plants induce shooting and finally flowering quite rapidly under long days; and sometimes even under short days but then much more slowly. Neither Wellensiek's phenomenon, nor flowering after defoliation as in henbane, could be observed in any of these plants (42, 201).

In *Oenothera suaveolens, O. longiflora, O. stricta,* and others the chilling requirement is not obligate. After a long delay, flowering may occur under long days without previous chilling. Other species are annual without any vernalization requirement, but they do require long days; still others that are annual are almost day-neutral. Up to now, grafting has not succeeded in inducing flowering by nonchilled plants that have an obligate vernalization requirement.

Celery (*Apium*) requires vernalization for flowering; as a result, active leaf growth occurs during the first year of culture. Exposure of young plants to chilling at 4° to 10°C. for a few weeks, followed by a return to warm temperatures, causes flowering within the first year of culture. Long ago it was shown that when the collar alone is chilled, with the main root remaining at the warm temperature, vernalization was effected, whereas chilling the root alone had no effect at all. It was one of the first examples (1930) to demonstrate that vernalization is sensed by the vegetative growing point, not by leaves or roots (54).

Carrots require different degrees of vernalization depending upon the variety of carrot in question (132). Recent Russian studies (6; 133 to 135; 219, 221) indicate that:

(*a*) Varieties from southern U.S.S.R. (Armenia and Daghestan), after the wet seeds are chilled at 2°C. for 50 to 80 days, have about one-third of the individuals flowering the same year.

(*b*) In varieties from more northern countries, seed chilling first seemed totally ineffective; but the following winter, after storing the isolates in a cool cave and then setting out in a hot greenhouse to accelerate growth, the plants from chilled seeds started initiating flowers two weeks later, but the others did not, suggesting that seed chilling had achieved part of the vernalizing process which would have been completed during further storage at a cool temperature (219).

(*c*) Chilling carrot isolates from a first crop causes complete vernalization of whole plants as well as of those from which the bud has been separated from the tap root after chilling and then planted alone. By comparison, when the bud has been chilled after removal of four-fifths of the root, the plant is not vernalized. The sugar-supplying root seems to play the same part here as does endosperm in the vernalization of cereal caryopses. The same fact had been reported for beets (134).

(*d*) Nonvernalized carrots grafted to flowering carrots or to an annual umbellifer ready to flower under long days (the dill) flowered under these

conditions (133, 135). Gibberellin may induce bolting and flowering without chilling (143).

Digitalis purpurea has long been recognized as requiring vernalization followed by long days to achieve flowering (5). The vernalization requirement still seems obligate; but the long-day requirement is less mandatory, at least in certain strains where I observed flowering after vernalization under short days. Wittwer, Bukovak *et al.* (280) showed that gibberellin causes these plants to shoot and flower without vernalization. I also observed this and showed that flowering is rapid in long days with frequent flower anomalies and the occurrence of perched rosettes on some axillary buds; but gibberellin also causes these plants to flower very late under short days (43). The other *Digitalis* species have various chilling requirements.

Scrofularia vernalis is a remarkable plant in many respects. It has a mandatory cold requirement. It is not seed vernalizable but natural chilling or artificial chilling, although less easily done, achieves vernalization of young rosettes as well as older ones, even those several years old. But after vernalization this plant is absolutely day neutral, proving that a long-day requirement is not necessarily linked with a mandatory vernalization requirement, as was often thought. Moreover, gibberellin causes considerable elongation of stems, even those of very young rosettes; this phenomenon is often associated with tumors issuing from cell proliferations in the medulla and cortex. Axillary shoots elongate too, but no evidence of flower initiation could ever be observed from such treatments. There appears here to be a sort of incompatibility between elongation caused by gibberellin and flower initiation whereas shooting after chilling is always followed by flowering (39, 41, 42, 43, 123).

Numerous other plants have been studied (38, 39, 43, 47). Many campanulaceous plants have a mandatory vernalization requirement, for example *Symphysandra, Campanula persicæfolia* [as was shown by Wellensiek (273)], *C. alliariæfolia, C. primulæfolia,* etc. In the last two, gibberellin has no effect either on shooting or on flowering. *Reseda luteola* requires extensive chilling, but gibberellin causes it to shoot and finally flower provided that it is injected into the stem at abnormally high concentrations (43). *Isatis tinctoria* absolutely requires vernalization and gibberellin injections can only make it shoot with difficulty. *Lunaria biennis* [studied by Wellensiek (271, 273)] has an absolute chilling requirement that diminishes with age, it is not seed vernalizable, and does not respond to gibberellin. This is also true for *Eryngium variifolium* (47) which, furthermore, doesn't grow at all when treated with gibberellin.

Several plants are remarkable because of the important modification exhibited by leaves after the chilling treatment. *Cichorium intybus* (274), the bitter Chicory, is seed-vernalizable (two weeks at 5°C.) [studied by Hartmann (111)]. After vernalization, the leaves are hairy and more serrated, they stand erect rather than spread out, and the plant flowers under long days with the new leaves sensing the photoperiod.

Crepis biennis and *Senecio jacobea,* which absolutely require vernalization, at least as rosettes, show similar modifications (39, 43, 47).

These examples could go on without end. The preceding are sufficient to show that the vernalization process is much more complex and varied than suggested by the comparison between classical winter rye and black henbane.

Vernalization of Caulescent Biennial or Monocarpic Plants

Contrary to rosette plants, caulescent plants form internodes of various lengths from the very beginning of growth. Their flowering is not associated, therefore, as in rosette plants, with the deep change which represents the shift of a brachyblast into a long stem. Several remarkable plants of this category require vernalization.

Euphorbia lathyris.—This species is remarkable for its regular elongation. It forms internodes about one-half cm. long under short days, and 1 to 2 cm. long under long days. As long as the plant has not been chilled, it keeps growing regularly producing stems that can reach two or three metres in length or more. Some individuals with different genetic factors finally flower under these conditions when quite old; but most of them do not flower except after chilling. Natural or artificial chilling, whether applied to young or old plants, causes vernalization. After returning to a warm temperature, there soon occurs the formation of one apex flower and, by the subterminal branching of the shoot, the inflorescence. After vernalization, flowering is strictly day neutral. Gibberellin applied to nonvernalized plants causes an initial rapid elongation of internodes, which then returns to normal a few weeks after the end of the treatment. This is repeated with every application of gibberellin, but no flowering is induced (43).

Various species of Iberis, particularly intermedia ssp. Durandii.—After the initial growth the nonvernalized plant branches into numerous very leafy shoots that never flower under long days until the plant is chilled. After chilling and returning to a warm temperature, flowering occurs equally soon under short days as under long days, but there are many more flowers formed under long-day conditions. If continuously grown under short days, without chilling, a few flowers appear after a long time. This is another example of those intriguing relations between short days and the vernalizing effect of chilling. Gibberellin has no effect on flowering even though it is very effective in increasing elongation (43).

Cabbages (Brassica).—Cabbages are botanically and physiologically highly variable. Their various chilling requirements have been extensively studied from an agricultural and horticultural point of view (52, 71, 78, 129, 180, 200, etc.). Certain species or varieties are rosette plants with tap-roots; for example, turnips, in which shooting and flowering are accelerated by seed vernalization, and "choux-navets" and rapes "rutabagas" which obligately require vernalization of leafy plants before initiating

flowers. By contrast, other stay caulescent; such as the fodder strains of *Brassica oleracea,* or the oil-seed cabbages (*B. campestris*) "Colza" and "Navette" which are annual strains with no vernalization requirement, and strictly biennial strains which obligately require chilling of leafy plants. Others are caulescent first and then form perched rosettes with numerous leaves. Among these are the ordinary garden cabbages (*B. oleracea capitata*) that show various chilling requirements depending on the strain. Gibberellin is an effective substitute for chilling in certain strains, sometimes causing an extensive elongation and occasionally flowering, but it is ineffective in other cases. A natural gibberellin-like substance has been found in the young floriferous shoots of certain cabbages (153). There are biennial cabbages that flower without chilling after grafting on flowering cabbages (135) or on annual mustards or on annual *B. crenata* (55).

Brussel sprouts (*B. oleracea gemmifera*) were studied by Stokes and Verkerk (249, 260). They showed that if this plant cannot flower without chilling it is not because of a real vernalization requirement, but because flowering cannot occur in ordinary warm or mild conditions. Ripeness to flowering appears after a juvenile stage and requires 30 nodes. From then on, exposure to cold temperatures of 5 to 10°C. induces flowering. Exposures taking place at such temperatures therefore initiate flower primordia and are not a "preparatory" process as is vernalization. Chinese cabbage (or Pak-choi: *B. sinensis*) seems to be similar (286). White mustard (*Sinapis alba*) is an annual plant in which wet-seed chilling slightly speeds up flowering (59). Indian mustard (*B. juncea*) shows a higher vernalization requirement which may be satisfied by seed chilling; immediate heat devernalization is not effective (238) as is true of cereals.

Many other cases of vernalization requirements of various degrees have been reported for other crucifers and in particular for radishes (61), *Lunaria biennis* (271), stocks (*Matthiola incana*), *Erysimum perofskianum, Cheiranthus Allionii,* the Siberian wallflower, etc. (79, 203, 273, 274).

Tomatoes.—Tomatoes are considered typical annual plants with a remarkable responsiveness to daily photo-thermo-periods. However, they display some noticeable phenomena related to vernalization (93, 124): the stimulating effect of wet-seed chilling has been reported by Russian authors particularly e.g., 10 days at 0°C. or 14 days at alternating temperatures of 18°C. and 0°C. in relation to crop precocity and yield (253, 254, 265). In other cases, particularly in Ailsa Craig and Potentate, other authors (18, 281) showed that wet-seed chilling had no effect. After the cotyledons open, however, if the seedling is exposed to 8°C. for nine days instead of being maintained under warm conditions, the first inflorescence will be formed one or two nodes earlier. When chilling is extended for a few more days, this first inflorescence will contain more flowers. Then, by successive periods from week to week or more, similar chillings stimulate the earliness of further inflorescences. Nightly cold temperature is mostly effective dur-

ing this kind of vernalization (13, 261), and for tomato growth generally.

Annual legumes.—Numerous cases of vernalization have been reported in caulescent annual legumes for example: sweet peas (125, 239), vetches (160, 239), lentils (130), chick peas (122, 194 to 196, 239), broad beans (240), etc. In many of these plants and particularly in vetches (239) the treatment increases the rapid growth of lower axillary buds, gives a more branched habit to plants, and makes them flower earlier.

Finally, the garden pea (*Pisum sativum*) reveals most interesting phenomena; the first flower is formed on a precise, genetically defined node for each variety (114). The next lower nodes bear aborted shoots that have a tendency to flower. Lower nodes later form the axillary leafy shoots. Often, dwarfed early varieties will generally present their first flower at a low node, the seventh for example, and are strictly indifferent to chilling (193). By comparison, late varieties that flower on higher nodes (the tenth or seventeenth for example) as Zelka, Unica, Greenfast. Alaska, Téléphone, etc. are markedly influenced by the chilling of recently soaked seed (62, 117, 121, 197). Exposing plants to a few days of 3° to 5°C. temperatures prior to growing them under normal conditions induces the appearance of the first flower 1, 2, or 3 nodes lower than in controls. Leopold & Guernsey (150) have shown that this effect is increased by soaking the seeds destined for chilling in an extremely diluted auxin and thiamine solution and that the effect increases with concentrations ranging from $10^{-7}M$ to $10^{-4}M$; but triiodobenzoic acid increases the vernalizing effect at a concentration of $10^{-7}M$, whereas at higher concentrations it reduces or inhibits the chilling vernalizating effect. After vernalization by chilling, even when enhanced by these chemicals, exposure to 39°C. for 18 to 24 hrs. totally removes the vernalizing effect. The same thing happens after a total lack of carbon dioxide for at least 1 hr. immediately after the vernalizing treatment. The carbon dioxide appears to be necessary to fix the vernalizing effect.

Highkin (117) showed that if seeds were exposed to 20°C. for some days after soaking and then subjected to chilling instead of being directly exposed to chilling immediately after imbibition, the vernalizing effect of chilling was completely prevented by a 5-day exposure to a warm temperature. He further showed that if the water used to soak pea seeds for 25 days at 4°C, was then used to soak other pea seeds which were immediately planted without chilling, it caused a significant reduction in the age of the appearance of the first flower (116). It was reported that this could also be obtained with the soaking water of chilled winter rye seeds and that these various soaking waters could speed up flowering not only in the pea, but also in other vernalization-requiring plants such as winter cereals. The active substance could be guanosine or something similar (118).

Gibberellin also effects the nodal placement of the first flower in peas. It greatly increases the internode elongation of dwarf varieties (this effect

was sometimes called "vegetative vernalization," a possibly erroneous usage). More & Bonde (17, 181) showed that on late dwarf Téléphone peas, gibberellin applications delayed flowering slightly by bringing the first flower node to a somewhat higher rank. When seeds from this variety are chill-vernalized, the rank of the first flower node is lowered; but if gibberellin is applied at the same time as chilling in the soaking water, it prevents the chilling vernalizing effect. When it is applied immediately after chilling, it destroys the effect of chilling and corresponds to a "reproductive devernalization."

Paton & Barber (198) and Haupt (114) showed that grafting a late variety on an early one, or even on a flowering late variety, causes an accelerated scion flowering by lowering the rank of the first flowering node. This effect occurs even though the scion is set with its own cotyledons and leaves. However, the effect is stronger when the scion is defoliated. The stock used as donor is much more effective if it keeps its own leaves but it is still effective if set in interstock, the stock being from a late variety. These observations show that the fixing of the first flowering node in the pea depends mainly on a stimulating substance, but that an inhibiting substance may also occur, and that the final result may come from a balance between both effects.

Vernalization of Perennial or Polycarpic Plants

The vernalization requirement in perennials has often been cited incidentally (23, 188, 199). However, the association of perennial growth (= polycarpism) and a vernalization requirement appears contradictory. In a plant that requires vernalization, the effect of winter cold is transmitted to the buds arising from the treated bud; therefore, when spring arrives, all of the buds should ultimately produce flowers and the plant should be monocarpic. Since the plant is perennial, one of the following alterations of the normal vernalizing process is likely to have occurred (42): (*a*) some of the buds are devernalized during summer; (*b*) some of the buds are not vernalized during winter chilling; (*c*) vernalization is not indefinitely autocatalytic among the second generation of buds derived from adventitious buds arising in the tissues that were not meristematic at the time of vernalization. Let us see what is the result of studies, especially those of the English authors with *Chrysanthemum* and of the French authors with various genera such as *Geum, Scabiosa, Teucrium,* etc.

Perennial plants in rosettes.—In the vegetative stage, *Geum urbanum* and one other *Geum* form short rosettes consisting of leaves without stipules; flowers occur on long axillary stems bearing leaves with stipules. The plants remain perennial by a rosette main axis which seems absolutely protected against all possibilities of flower formation. We showed [Chouard (43, 47), Chouard & Weber (50), Lé (149), Weber (269)] that before the autumn

cold, no axillary bud had yet been transformed into an inflorescence primordium. The normal chilling time for vernalization is at least five weeks, at + 1° to + 4°C. This chilling confers a flowering capacity to the buds located in the axil of the younger visible leaves and some of the still younger leaves. Everything occurs as if the youngest, barely differentiated buds were indifferent to chilling vernalization. They react to vernalization as they become more differentiated; finally, as they grow older, their state is definitively fixed, either in the vegetative stage if they have not been vernalized in time, or as inflorescences if they have been vernalized in time.

Older axillary buds on the outer part of the rosette generally stop growing because of the correlative inhibition exerted by the active terminal bud. If the latter is taken off, outer axillary buds start growing—but in rosette only. Then, if they have undergone chilling, those among their own axillary buds which were responsive at that time and were vernalized, flower.

When the vernalizing temperature is below 1°C. no initiation appears during the minimum chilling period, but then, after warming up to 4°C. to as high as 30 to 35°C, the evolution of vernalized buds into inflorescence primordia followed by the shooting of these inflorescences and their flowering occurs slowly. The process occurs more quickly at cool temperatures and at the normal rate at warm temperatures. Hot temperatures cause accelerated secondary branching. Vernalization occurs around 4°C. and is immediately followed by a slow tissue differentiation which goes on actively even at such a low temperature.

The most easily vernalizable buds are those which, at the time of the chilling treatment, consist of their own apex meristem with a few roughly differentiated leaf primordia. If the chilling time is extended to 10 to 15 weeks, more buds are vernalized, particularly those that were too young at the beginning of the treatment. If the treatment is prolonged for 30 to 50 weeks, a remarkable phenomenon occurs : the terminal apex itself, as well as the older outer buds of the rosette, is vernalized. It follows that all the existing buds become vernalized. They form inflorescences after a return to warm temperatures and also form a terminal inflorescence (which never occurs in any *Geum* in any natural climatic condition of the world). These polycarpic plants become monocarpic and die after complete flowering of all their buds.

By taking off a leaf with its axillary bud and planting it, the bud can be freed from correlative inhibition and reveal whether it is vernalized or not. Inflorescences then form several leaves with stipules before ending in a flower at their apex. Without exception all axillary buds of an inflorescence are vernalized and able to flower. In a few exceptional cases, we could obtain adventitious buds on the petiole of leaves with stipules on flowering shoots. These newly formed buds are not vernalized and only give rise to rosettes.

Flower initiation, growth, and flowering following vernalization are

absolutely day-neutral. We could never obtain devernalization either by short-day treatment or by a high temperature (35°C.) even when applied immediately after chilling. The continuance of the vernalized state in an autocatalytic manner in all successive generations of buds of inflorescences is indefinite. Cutting young flower buds or treating them at a high temperature (30 to 35°C.) induces considerable axillary branching in the inflorescence yet all successive generations of buds still can flower. Shifting non-vernalized plants from short days to long days does not cause any flowering (that is to say this plant does not show Wellensiek's phenomenon).

Up to now, gibberellin has not caused *Geum* to flower. In the absence of chilling gibberellin induces the shooting of some axillary buds or even of the terminal bud, which then forms an erect leafy stem. Leaves take on some of the form of flowering shoot leaves but do not have stipules. In spite of repeated gibberellin treatments, these elongated shoots finally form perched rosettes.

Certain individuals 1 to 10 per 1000 at the most) in *Geum urbanum* show a reduced vernalization requirement. These individuals grow one or two inflorescences without chilling, after 1 to 3 years of growth in warm temperatures. They may sometimes be induced to flower by gibberellin. They seem to belong to physiological races which differ genetically from the common type. In *G. bulgaricum* and *G. intermedium* this situation is true of most individuals, whereas in such species as *G. canadense, G. album, G. macrophyllum,* etc. we have only observed up to now a mandatory vernalization requirement, similar to that generally found in *G. urbanum.*

In *Geum,* mature achenes are dormant due to tegument inhibitions. Excised mature embryos are not dormant. Neither achenes nor excised embryos have yet been chill-vernalized. But young seedlings having only two small leaves in addition to the cotyledons can respond to vernalization and can flower on a young axillary shoot.

Geum, and especially *Geum urbanum,* must be considered as a new type of vernalization-requiring plant groups, like Petkus rye and henbane, which were the first two classical types.

Scabiosa succisa (=*Succisa pratensis* = *S. praemorsa*) behaves much like *G. urbanum* particularly in relation to the permanent inability of the terminal bud rosette to flower. Normally, vernalizing chilling induces the ability for further growth into an inflorescence in the two main axillary buds at their very young responsive age. Here however, under long days, after a sufficient delay for differentiation, inflorescences grow rapidly with long internodes and then flower. Under short days, the phenomena are slower, internodes are shorter, and flowering occurs much later. After vernalization, this plant therefore is a "quantitative long-day plant."

We first used plants from the "forest of Breteuil," in Perche. All plants showed the following remarkable reactions. When grown under short days at a warm temperature up to the age of a vigorous rosette the plants, when

placed under long days at warm temperature, soon flowered as if vernalized, thus reproducing "Wellensiek's phenomenon" as found in *Campanula medium*. Moreover, repeated gibberellin treatments induced the shooting of axillary buds as if they were vernalized. Under these conditions, they flowered either slowly with short days or faster with long days and finally attained about the same length. Experiments carried out on individuals from other countries, such as Burgundy and the forests of "Brie," showed much greater vernalization requirements. In these races, gibberellin induced temporary shooting as in *Geum*, but no flowering, and Wellensiek's phenomenon did not occur. We have separated different clones from different individuals of the same population: each one behaves consistently but may differ physiologically one from another. Again this example shows how different the behavior of different strains of the same Linnean species can be (35, 42, 45, 47, 115).

Perennial plants in rosettes with terminal inflorescence.—Contrary to both preceding types, the following plants normally flower after transformation of the main axis into an inflorescence. These perennials, which require vernalization of various degrees, are probably more numerous than formerly thought. Many perennial meadow Graminaceae, studied by Cepikova (23) and more recently by some others (53, 168, 199) but mainly by Wycherley (285) initiate inflorescences on main shoots after chilling. *Cynosurus cristatus* requires 15 to 30 days at 5°C. or less and active flower initiation may follow, even at such low temperatures. It is markedly speeded up by long days; returning to short days can force some of the inflorescences back into rosettes. *Lolium perenne* is easily vernalized by only 15 days at 5°C. *Dactylis glomerata* requires a much longer chilling period. *Poa pratensis, Dactylis glomerata,* as well as a strain of *Cynosurus* and probably *Agropyrum repens* seem to be vernalizable but on shoots brought into a state of short and stout shoots by a short-day treatment (53, 88, 188, 195, 199, 285). I also observed mandatory or substantial vernalization requirements in *Poa supina,* various *Festuca, Bromus,* etc. (47). Their behavior seems to display significant differences from clone to clone and will soon need careful investigation, especially the requirement for short days before or during chilling.

Saxifraga rotundifolia obligately requires vernalizing chilling; the subsequent flower initiation is photoperiodically day-neutral, but the length of the flowering shoots and the number of flowers are much greater with long than short days. Many perennial Crucifers behave similarly, except for certain rosette types, e.g., *Draba aizoides* and *D. hispanica,* which normally grow as small rosettes with numerous pointed leaves. These must be vernalized and then they initiate flowers even at relatively low temperatures and finally flower irrespective of the photoperiod. When maintained constantly in a warm temperature, the rosettes grow a short, sometimes flexible stem with numerous leaves, that never bears flowers. Here gibberellin increases

elongation without inducing flowering. It does not appear that the axillary buds can be vernalized by chilling. In the genus *Erysimum,* numerous perennial species also require vernalization, either obligately, or almost so. Without chilling, certain species remain as mere rosettes while others, especially at warm temperatures, grow extensive leafy stems of various lengths. Gibberellin generally increases elongation without inducing the flowering that occurs normally after vernalization irrespective of the photoperiod. *Dianthus,* which we will refer to again, can be classified here. Various species of *Primula,* many of *Potentilla, Agrimonia, Lychnis coronaria, viscaria,* and *flos-cuculi, Cardamine amara,* various species of *Silene, Scrofularia alata,* etc. under observation for numerous years, proved to have mandatory vernalization requirements. In *Eryngium variifolium,* the vernalization requirement seems important and gibberellin has been totally ineffective in causing even the least elongation or modification of the plant habit (43, 46, 47).

In *Scrofularia alata,* a perennial absolutely requiring vernalization, gibberellin induces considerable elongation under either long or short days, but no flowering. However, after an insufficient period of chilling that has no apparent effect, gibberellin induces both elongation and flowering. The small seeds may be vernalized, contrary to those of henbane which cannot be (148). When fully studied, *Scrofularia alata* could become the other exemplifying type for perennial rosette plants requiring vernalization.

Scabiosa canescens (= *suaveolens*), after germination, produces simple obovate leaves that spread flat on the ground. After chilling, newly formed leaves are deeply pinnatisect into fine segments. The plant then grows and flowers quickly with long days, slowly under short days. Without vernalization, plants grown from seeds from an apparently homogeneous natural population display great genetic variability. Some plants of this category remain in rosettes for years with simple young leaves; others grow longer after a certain time lag but retain the simple leaves on a long stem for prolonged periods; others spontaneously form divided leaves quite early while growing either slightly or extensively and finally flower late. This plant, perennial according to the nonvernalized or devernalized shoots with simple leaves formed at the base, illustrates very well the leaf polymorphism that follows vernalization as was reported for bitter *Cichorium.* It also shows that the elongation and the different leaf forms that necessarily precede flowering, can either be linked or separate. Therefore, they are possibly independent phenomena (43, 47).

Caulescent perennial plants.—Before vernalization, certain of these have a short stem, differing little from an elongated rosette. *Dianthus* (carnation) (43, 47), displays all possible types of vernalization requirements: none in *D. superbus* which keeps flowering again and again under long days from bottom sprouts; low in *D. gallicus, seguieri, campestris,* etc. which elongate into creeping or stoliniferous stems and flower quite abundantly, but earlier

and still more abundantly after vernalization; and important in *D. arenarius, attenuatus, geminiflorus,* and *caryophyllus,* which develop short stems of which the strongest shoots produce 1 or 2 flowers after 2 to 4 years of growth under warm conditions. In addition, the requirement is mandatory for numerous species with rare individual exceptions: *D. deltoides* forms long, very leafy shoots that never flower without vernalization or else flower very late and poorly; *D. coesius* and *D. carthusianorum,* remain as very short stems with heavy masses of leaves; *D. neglectus* and *graniticus* (with slightly longer stems) and *D. squarrosus,* with long stems, never flower without vernalization. *D. barbatus* (268, 271) forms stalks with very dense leaves and must be vernalized except for one strain that keeps flowering constantly and which, through a graft made by Waterschoot (268), induced a vernalization-requiring plant to flower. Gibberellin appears not to induce flowering in carnation in place of vernalization. Cultivated carnations (from *D. caryophyllus*) have more moderate requirements for flowering, e.g., everflowering carnation (14) must have formed 9 to 11 pairs of leaves before it can be vernalized. When chilling is then supplied, they can flower under long days at the fourteenth node. Flowering is markedly later under short days or without chilling.

Many *Leucanthemum,* mainly *L. cebennense* (= *monspeliense*) (43, 47), and probably *Pyrethrum cinerariaefolium* (91) form short bushy stems that only bear flowers on the terminal axis after vernalization under essentially day-neutral conditions. However, the pedicels are very short under short days and very long under long days. In these plants, as in carnation, very young or insufficiently robust shoots at the time of chilling are insensitive to vernalization and thus ensure the perennial condition.

We also find typically caulescent perennial species (42, 43, 47), *Teucrium scorodonia* is one of the best examples, forming suckers and leafy shoots. It never flowers unless previously vernalized. After vernalization it requires long days, although not always. The terminal shoots terminate in inflorescences. The secondary shoots, leafy lower sprouts, and subterranean stems or suckers maintain the perennial condition. Repeated applications of gibberellin can induce an elongation of several metres, but no flowering. *Teucrium scordium* continues in the form of creeping shoots without flowers when nonvernalized (47).

Many woody plants (bushes or even trees) have similar requirements (47), e.g., *Medicago arborea,* in which a chilled twig flowers without this ability being transmitted to the other nonchilled twigs of the same plant. Wellensiek also showed vernalization requirements for the woody crucifer *Cheiranthus cheiri,* the wallflower (273, 274). I found *Alyssoides* (= *Vesicaria*) *utriculatus* to also require vernalization. Obligate vernalization requirements are also found in various species of *Cistus,* in *Salvia* (*officinalis, triflora, lavanduldefolia*), in *Lavatera* (47), etc. It is also very likely that large trees such as the olive tree (*Olea europea*) have varying vernalization requirements depending on the variety (4, 110).

In other species, such as *Geum* and *Succisa,* the apical bud is protected against vernalization, but they are typically caulescent. An example is *Anagallis tenella,* which covers the ground with stoloniferous stems, mandatorily requires a long vernalization and then the axillary buds flower only under long days. Vernalization does not seem to extend to axillary buds other than those responsive at the time of treatment. Radicant *Lysimachia nemorum* and non-radicant *Saxifraga hypnoides* behave similarly (47).

Chrysanthemum hortorum or *morifolium,* the garden chrysanthemums (33, 49, 203) flower in short days more or less obligately (late varieties) or only prefer short days (early varieties). Some of them (22) flower irrespective of temperature; others are stimulated by warm temperatures ($> 12°C.$) and others by chilling ($< 12°C.$). Although short days induce flowering, chrysanthemums also require various degrees of preparation. I have already shown (33) that certain varieties respond to short days much earlier and more effectively when they have previously received a long-day treatment. Schwabe (230 to 232) showed that certain varieties require a preparatory chilling period of various extents which can be considered as vernalization. The treatment is more effective when given at night and for about three weeks around 4 to 6°C. These phenomena have also been carefully studied by Vince (262, 263) who showed in particular that only certain varieties may undergo the preparatory phase partly with cold temperature treatment and partly with long-day treatment. Many cold-requiring varieties grow in warm temperatures as creeping shoots, forming short sprouts with layered leaves, as in rosettes.

Removing the rosette terminal bud in certain of these releases axillary shoots that are then directly influenced by short days (233, 234, 263). Most often, axillary buds are vernalized simultaneously with the terminal bud, but not always down to the base of the stem. Moreover, exposure to high or low light intensities, or growth in the ground (suckers) causes devernalization, which ensures the perennial state. This is most effective at temperatures less than 18°C., even if the plants receive additional soluble sugars. However, heat does not devernalize in high light intensities. The vernalized state is not graft transmitted. Gibberellin often suppresses the branching stage, but the long shoots still develop flowers in a few varieties, even under short days. Picking the flower buds formed under short days sometimes induces flowers directly on young suckers, but this is not true for most varieties. The varietal responses are so varied that the interesting investigations recently completed are now giving rise to further studies [cf. recent works, Cathey (22), Chan (29), Doorenbos (72), Schwabe (230 to 236), Vince (262, 263)].

MECHANISMS OF VERNALIZATION

Up to now we have reviewed the main experimental work that determined the characteristics of vernalization in various species. We will now examine what processes were inferred in attempting to rationalize the mechanisms involved.

The Stadial Development Theory and the Reversibility of Vernalization

From embryogenesis to flowering, a plant grows through successive stages. The identification of these stages is useful in further analyzing every mechanism in their ontogeny. The "stadial theory" however, is exacting; it holds that the same stages occur in all plants and that they are fundamentally irreversible. For further information refer to Lysenko who proposed the theory (155 to 159) and to commentators on the theory (279). If these stages are believed to be ontogenetic natures or properties defined according to an *a priori* postulate and unaltered by experiment, they are to be considered as expressing a faith that does not fall within the scope of scientific experimental analysis. If we hold to mere facts that can be repeated in experiments we speak another language. We indeed recognize stages in plant development, but these are neither universal nor irreversible. For example, we cannot say that the vernalization process, or "thermostage," is universally imposed merely as a temperature-controlled period of development. *Bidens radiatus* reacts to photoperiods as soon as its cotyledons turn green, without any previous thermostage (37); also, the "photostage" is not always necessary for flowering. Flowering primordia exist in mature peanut seeds before any light treatment of the seedling. When a thermostage is required, it may be experimentaly reversible in certain cases, as we have seen for experimental successions of devernalization and revernalization. What is the obligate requirement for new vernalizing chilling if not the need for a new thermostage, the former having been fully eliminated? Bud regeneration returns the plant to most of its juvenile stages. In experiments with proliferating flowers (44) the partly differentiated floral apex is reversibly returned to a vegetative apex of a shoot which again will have to go through all the previous stages before flowering. As far as the words are concerned, we prefer to consider purely empirical stages as observed for each species in our experiments and their reversible or irreversible sequence, and to analyze the various regulating mechanisms, whether independent or correlated, that control the sequence of the development.

Hereditary Transmission of Developmental Mechanisms

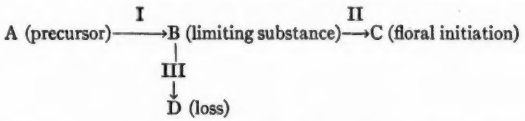
A requirement for vernalization is an hereditary property of certain species and concerns the transmission of a physiological characteristic. We have seen that a single species often includes several strains that display various kinds of vernalization requirements. We even found wild species that seem to have a simple vernalization requirement but in experiments reveal that they are a mixture of mutants with differing requirements. When hybrids can be obtained from plants with different vernalization requirements, the studies (169, 207) have shown that the vernalization characteristics are borne by a few Mendelian factors in which the various alleles are often selected by an adaptation to climate, e.g., one gene only in the case

of the two black henbane strains. But vernalization was used as a stepping stone to demonstrate the transmissibility of acquired characters. Mitchourin's followers state that the growing of spring wheat in the autumn wipes out the inheritance of the "spring" character after a severe winter of growth; from the seeds of the few surviving individuals a second autumn crop would show a new "winter" heredity instead of the "spring" one (158, 159). In certain countries, vernalization is mostly studied from this point of view. Since such experiments have not been successfully repeated in any country where properly controlled experiments and pure strains were used, and since they do not concern the physiological processes involved in vernalization, there is no need to discuss them here.

However, the stadial theory led several physiologists to study so-called "lasting modifications" that were found to be reversible in a few generations. For example, Séchet (239) reports the "remanent effects of vernalization" in *Camelina sativa, Cicer arietinum, Vicia faba,* and in oats. Highkin (119) reported a nonidentical but related phenomenon in the training of peas for surviving detrimental thermal treatments.

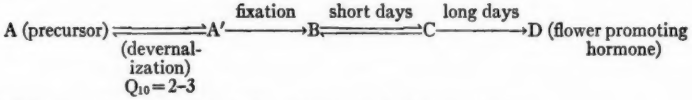
Descriptive Formulations of Vernalization

Several authors have established formulation of the facts observed. Lang & Melchers (144) express their observation on henbane as follows:



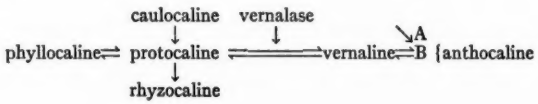
I occurs in the cold, II takes place at normal temperatures and in the presence of oxygen, III is heat devernalization without oxygen, B is not necessarily the precursor substance of C, but may also be an agent involved in the synthesis of C.

Gott, Gregory & Purvis (94) and Purvis & Gregory (214) having corrected their previous formulation (212) of vernalization in rye Petkus now represent it as:



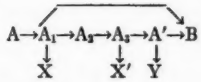
which contains in brief all the above-cited observations.

Van de Sande Bakhuyzen (9) expresses his findings by the following formulation:



A "thermophase" occurs under cold conditions releasing an enzyme "vernalase." Then during an "interphase" with warm temperatures and possibly short days, vernalase results in "vernaline" which, in a final "photophase," gives rise to "florigen" B; the latter, according to the proportions in which it combines with photosynthetic products A, either does or does not induce flowering. This diagram is meant primarilly to express simultaneously the mechanisms of short- and long-day plants.

Napp-Zinn (186) groups his studies on *Arabidopsis thaliana* "Stockholm" strain in the following diagram:



in which A is a thermostable precursor; A_1 is the first thermolabile intermediate that can be shifted toward X by a heat treatment at 30°C. soon after sowing and causes anti-vernalization; A_2 is a thermostable intermediate demonstrated by the possibilities of revernalization after five days of devernalization; A_3 is the second thermolabile intermediate, demonstrated by the partial shifting towards X′ at 20°C.; A′ is the third thermolabile intermediate demonstrated by devernalization at 30°C., leading to Y, and corresponding to the thermolabile intermediate of Purvis and Gregory; and B is the final thermolabile intermediate of vernalization, leading to flowering. It may be directly attained from A by a shunt at 20°C.

These formulations are handy to memorize and they stimulate further investigations on hypothetical substances; but they provide no more clarification than the authors' descriptions of their own results. Further, they may require adjustment for each new discovery and they also change for each plant type that behaves in a particular way and does not fit the particular representation.

Regulatory Mechanisms of Development

From a simpler point of view, I think that the experiments permit us to analyze separately some mechanisms found either naturally separated or interrelated. For example, the complex mechanism of vernalization is not always linked to that of photoperiodism. Table I shows that among the various species of plants there are all possible combinations of various vernalization and photoperiodic requirements (see Table I) (34, 36, 38, 39, 41, 42).

Before constructing a general theory of reproductive development—if one is possible—we have to analyze carefully one after the other, every elementary mechanism involved in it and choose the best possible example of each mechanism where it constitutes the limiting factor. Then we will consider the various relationships between these mechanisms. For example, as we have just seen, vernalization may be either independent from or associ-

TABLE I

Examples of the Different Combinations of Several Regulatory Mechanisms of Development, Including Vernalization

Requirement for vernalization by chilling / Photoperiodic requirement	Obligate (qualitative)	Partial (quantitative) — Great	Partial (quantitative) — Small	None or absent
Hemeroperiodism (long-day) Obligate (qualitative)	*Oenothera biennis, parviflora, lamarckiana* *Anagallis tenella* *Dianthus graniticus, coesius*, etc. *Hyoscyamus niger* (biennial strain) *Campanula medium* (Wellensiek strain) *Iberis intermedia*	*Oenothera suaveolens, stricta, longiflora* *Dianthus arenarius*	*Dianthus gallicus* Spinach (some varieties)	*Scabiosa ukranica* *Dianthus superbus* *Anagallis arvensis* *Hyoscyamus niger* (annual strain) *Lavauxia (Oenothera) acaulis* Spinach (some varieties)
Great	*Digitalis purpurea*	*Scabiosa canescens*		*Nigella damascena*
Partial (quantitative)	*Dianthus barbatus* *Teucrium scorodonia*	*Dianthus caryophyllus*	(depending on variety) *Agrostemma githago*	*Scrofularia arguta* (apical stems with expanded leaves) *Dianthus prolifer* *Dianthus barbatus* (one strain)
	Scabiosa succisa *Cheiranthus cheiri*	Winter wheats and cereals	Alternative wheats and cereals	*Oenothera rosea* Spring wheats and cereals
Small	*Leucanthemum cobennense*		*Sinapis alba*	*Nigella arvensis*
None or absent (day-neutral)	*Scrofularia vernalis* *Euphorbia lathyris* *Scrofularia alata* *Geum urbanum, macrophyllum, canadense* *Draba aizoides* *Saxifraga rotundifolia* *Lunaria biennis*	Several *Erysimum* *Geum intermedium* *G. bulgaricum*		*Scrofularia peregrina* *S. arguta* (basal aphyllous stems) *Senecio vulgaris* *Euphorbia peplus* *Lunaria annus*
			Pisum sativum (some varieties)	*Pisum sativum* (some varieties)
Nyctiperiodism (short-day)	Summer flowering { *Chrysanthemum* ×*hortorum* Autumn flowering { (*morifolium*) (depending on the variety)	*Chrysanthemum* ×*hortorum* (=*morifolium*) (depending on the variety)		*Chrysanthemum* ×*hortorum* (*morifolium*) (depending on the variety)

Many other examples could be given, but the above are sufficient to demonstrate that all the different combinations may be encountered. According to varietal or strain differences, some species may appear in several squares of this table.

ated with photoperiodism. The partial or total replacement of chilling by short days to achieve vernalization is illustrated by the rye Petkus, *Campanula medium* (Wellensiek's strain), and by one strain of *Scabiosa succisa* but not by most other plants. Partial or total replacement of vernalizing chilling by long days is exemplified by certain *Chrysanthemums* and not others. Heat devernalization, obtained by warm temperatures applied immediately after vernalizing chilling, although very common, is ineffective for Chinese mustard and *Geum*. Late devernalization after fixation of the vernalized state seems impossible with the rye Petkus, henbane, *Geum*, etc. but seems most readily attained with *Oenothera* and beets.

The analysis of the substitution of chemicals, particularly gibberellin, for chilling in order to achieve vernalization shows, as we will see later, that the vernalizing process probably consists of several stages; and some of them may be absent in related strains.

After the broad eco-physiological analysis of vernalization presented in the second part of this review, there remains the imposing task of interpreting each elementary process at the physico-chemical level.

Common Properties of All Vernalization Phenomena

The variety of elementary processes must not overshadow the characteristics common to all vernalization phenomena. Some of these are ecological, others physico-chemical.

Properties related to environmental conditions required for vernalization (42, 98, 173, 174, 273).—We have reviewed these in detail for the rye Petkus and for henbane; with reference to other plants we can summarize the results here. Chilling between the extreme limits of −6° and +12 to 14°C., normally from +1 to +5 or 6°C., is the fundamental agent of vernalization. It is only effective when applied for several days, weeks, or months in the presence of oxygen to moist enough tissues containing enough carbohydrate to support adequate respiration. Concomitant growth may be either moderate or very slow; if it stops completely, vernalization does not occur. Vernalization is directly perceived by stem or bud primary meristems; it is sensed at a certain age which is very early (immature embryo) or much later (leafy plant) according to the species. It is transitted by more or less stable or prolonged autocatalysis or self-perpetuation to the buds arising from the vernalized ones. It consists in the attainment of a new functional ability (the flowering ability) that is later "revealed" by flowering under special conditions (long or short days, various temperatures, etc.) which are characteristic for each species and variety.

Physico-chemical properties of vernalized tissues (76).—A great many investigations have been devoted to this question which consists essentially in comparing treated tissues at the end of the vernalizing treatment with nonchilled tissues, either to check on the completeness and effectiveness of the vernalizing process or to try to observe the intimate nature of the

process. The second aim has not yet been fully attained, for in fact, we only observe either the ordinary consequences of growth in the cold or the metabolic consequences of vernalization itself, which are simply concomitant with the acquisition of the ability to flower and are not the agents conferring this ability (75, 76).

Thus, it is observed that carboyhdrates are depolymerized, resulting in an increase of soluble sugars, sometimes not very great (62, 63, 66, 75); depolymerisation of reserve proteins occurs with an increase in soluble nitrogen compounds (62, 65, 69); and there is a fall in the lipid content (64, 73 to 75). These are typical consequences of a low temperature metabolism and probably not specifically related to vernalization (38, 87). Moreover, during germination, a phase occurs in nonvernalized seeds in which the respiratory rate increases with a respiratory quotient approaching 1.0. In vernalized seeds, this phase is characterized by a low respiratory rate and the respiratory quotient only slowly increases to 1.0 (179).

Also observed are a reduction of the auxin level (57, 242), a shift of the isoelectric point of proteins toward acidity (283 to 286), a slight increase in the coagulability and permeability in the protoplasm (30, 83), and a modified pH of the cell liquids (214). After vernalization cells of the vegetative point are less readily coloured blue by ferric chloride and potassium ferrocyanide, showing, as well as by other colour reactions, a possible change of pH (11, 20). The serological properties of a vernalized plant do not seem modifled compared to the control (1, 2). Cholodny's former theory (32), involving a transfer from endosperm of auxin and other hormones such as a blastanin, is no longer entertained (112, 242).

Enzyme activities are markedly modified (57, 69, 112, 120, 224, 225, 227, 242) e.g., relative increases in the activity of sugar hydrolases, in particular amylase and invertase (yet there are contradictory results), and of phosphatase, lipase, catalase, etc. Recent Russian authors (75, 246, 247) emphasize the alteration occurring in enzyme adsorption on protoplasmic surfaces, with tendencies toward depolymerization after chilling, following a loosening of the links between enzymes and protoplasm. There is also a reduced activity of cytochrome oxydase and succinic dehydrogenase and increased activity of ascorbic acid oxydase and of malic and citric dehydrogenases (247), even though, in general, the overall respiratory rate is not particularly changed at the end of vernalization. Changes in the vitamins C and B contents take place during (239) and after (189) vernalization.

When vernalization is complete, the ensuing metabolism is soon similar to that of the control. However, certain authors think that the above enzyme modifications persist after chilling and that they are like the enzyme characteristics attained directly by nonchilled annual strains (246).

In addition, it was reported that after vernalization certain plants (radish) show either increased resistance (240), or decreased (239). Chilling by itself is a process of training a plant to resist frost (259). Vernalized

cereals respond more to water than do nonvernalized controls, but they also suffer more from drought (134). Their capacity for mineral and water absorption is changed (191) and the anatomical status looks more mature (25).

All these interesting facts still do not solve the problem: What is the biochemical nature of the process of vernalization?

Hormonal Transmission of Vernalization

I emphasized that the autocatalytic transmission of vernalization is restricted entirely to those meristematic cells derived from actually vernalized ones. We should not overlook the fact, however, that vernalization may also be transmitted as if it were effected by some substance diffusing from cell to cell. The Melchers and Lang graft experiments on henbane (168 to 172, 178) elicited much enthusiasm and were interpreted as pointing to some general process. In actual fact, examples of vernalization induction from donor to receptor, by way of a graft union are few in number. Most of these are listed by Lang in his 1952 Review (140). These are: henbane as a receptor for several donors, beets, cabbage scion upon annual *Brassica* or upon mustard stock, carrot upon dill and, more recently, late peas upon early peas and grafts of different strains of sweet William, but almost nothing more. In other cases, vernalization is strictly localized to the chilled part of the stem (47, 289). An hypothesis based on a mobile vernaline holds only for a few special cases.

Chemical or Biochemical Vernalization

Many authors understand "chemical vernalization" to mean the more or less synergic effect of some substance acting conjointly with the chilling of seeds to promote vernalization. In *Brassica* and in peas (in as much as vernalization here is truly a vernalization as defined earlier) indole-3-acetic acid synergizes chilling whereas gibberellin and 2,3,5-triiodobenzoic acid antagonize chilling (27, 150). See also the several cases of synergism between different chemicals and chilling reported by Séchet (239).

The hypothetical vernaline has never been extracted from plants (176), but some reports indicated that vernalized seeds may yield undefined substances exerting a vernalizing effect when used to soak unvernalized seeds. Purvis and Gregory reported that extracts from cereal seeds being processed for vernalization could replace the effect of chilling, but they have not reported again since 1953 (215). More recently, Highkin showed that if late pea seeds are chilled for a long time they yield water extracts that replace chilling for the vernalization of other peas or even for winter cereals (116, 117). Guanosine may be involved (118).

The problem of vernalization by chemicals has had to be reinvestigated completely since Lang's discovery of the effect of the prolonged gibberellin treatment of henbane (biennial strain). In the green house under long-day conditions, such treatment induces elongation and then flowering (142). This

report stimulated numerous experiments (15, 21, 43, 47, 153 to 156, 273, 280). The results are as follows. Most often gibberellin increases the elongation of the treated organ; caulescent stems become longer, "rosettes" elongate into leafy stems, etc. However, among plants requiring vernalization, few flower as a result of gibberellin treatment in the absence of chilling. Replacement of chilling by gibberellin is observed only among rosette plants mainly those flowering on elongating terminal buds such as: henbane, *Centaurium minus,* fox glove, *Oenothera lamarkiana* and *parviflora* (but not *biennis*), *Reseda luteola* (slightly), *Beta maritima* (but not several other *Beta*), some strains of *Scabiosa succisa* and *Campanula medium* (but not other strains), and a few species of *Geum* (*intermedium, etc.*). Likewise, gibberellin replaces the photoperiodic induction of flowering in some rosette plants *Samolus parviflorus* (146) and *Polemonium coeruleum* (43). In *Scabiosa ukranica,* under short-day conditions, gibberellin causes elongation and the formation of "inflorescence" primordia although not of "flower primordia." Usually, even repeated treatments will at most cause elongation from the rosette stage but without even initiation of inflorescence primordia (*Scrofularia vernalis, S. alata,* winter cereals). Frequently, elongation is restricted and results in either temporary or permanent "perched rosettes" (*Geum urbanum, Campanula medium,* sugar beets). In other plants gibberellin causes little (*Dianthus*) or no elongation (*Saxifraga rotundifolia, S. cotyledon, Eryngium variifolium,* etc.). Lastly, among caulescent plants, gibberellin has so far failed to replace the vernalization or photoperiodic requirements for flowering (*Euphorbia lathyris, Teucrium scorodonia,* etc.) (42, 43). Species vernalizable by seed chilling (*Brassica campestris, Cicer, Lens,* etc.) are not vernalizable by treatment of the seed with gibberellin (25).

Problems to be Solved

Having summarized what is already known about vernalization, we will now mention the many unsolved problems. The duration of the "juvenile period," i.e., the time elapsed before "ripeness to vernalization" is achieved must be determined in many cases. Most plants with an obligate vernalization requirement have embryos within the mature seed that cannot be vernalized (*Scrofularia alata* is an exception). This should be elucidated to find out whether chilling such seed effects a preliminary part of the vernalization process; also, a determination should be made as to when these plants actually become fully vernalizable.

Vernalization kinetics remain to be clarified. Further, the effects of overchilling should be investigated in as much as it may depress vernalization or conversely prolong the effectiveness unduly.

Anti-vernalization techniques should be elucidated in relation to the efficiency of chemicals (metabolic and growth inhibitors) about which we know very little (28, 48). In this regard, the efficiency of gibberellin as an

anti-vernalizant of pea, the effect of metabolic agents, deficiency of food such as sugar (99, 208, 210), and the role of physical agents [heating or leaching previous to chilling (112)], remain to be studied in more detail.

Devernalization should be "explored" in a number of plants which have not yet been studied from this point of view. The effect of devernalization treatments should be studied not only immediately after the vernalization process but subsequently, for instance as in the effect of short days on beets (164) or *Oenothera* (201, 202). This kind of devernalization is probably very different from the classical devernalization by heat or by anaerobiosis immediately after chilling. Alternating temperatures should be investigated as to whether they delay or accelerate the vernalizing process.

Among plants bearing leaves, the roles of intensity, duration, and quality of light during chilling appear important and deserve further study. Is light necessary just to supply sugar or does it have specific effect? We do not know for sure whether leaves formed before vernalization are or are not photoperiodically receptive. Data is still scanty as to the role of the root, which when tuberized, intervenes as a sugar donor.

Some data suggest that vernalization may proceed by successive stages that may differ both as to requirements and results, e.g., short chilling, unable by itself to promote vernalization, may become effective by an additional treatment with gibberellin, which by itself would not have been effective. This should be checked (148, 201).

What has been termed "vernalization" in chrysanthemum, tomatoes, and peas, may differ from what is termed "vernalization" in henbane and *Oenothera*. Peas growing from nonchilled seeds produce, below the flowering nodes, axillary buds which tend to develop into abortive flowers. Had the seeds been chilled, flowers would differentiate; in that case, the "assumed vernalization" completes what otherwise would have only been initiated. Yet to be clarified is the chronological sequence of the initiation of inflorescence, the initiation of flowers, the various stages of elongation, and the possible interrelations among these events.

Perennial plants deserve special attention in relation to all the problems alluded to above as well as those peculiar to themselves. How can the perennial condition be preserved? The various alternative methods discussed above have been investigated only for a very few plants.

New researches are urgently needed on the total or partial replacement of "vernalizing chilling" by gibberellin, other chemical agents, extracts from vernalized plants, or by long or short days. The vernalizing effects of short days (apparently complete or partial) when followed by long days (Wellensiek's phenomenon) or in the absence of long days (Petkus rye, *Iberis durandii*) require more research. We know nothing about the possible similarity to the above of the different photoperiodic requirements in amphiperiodic plants.

Lastly, the biochemistry of vernalization should be investigated with

increased vigor. Emphasis should be given to the identification of natural stimulating or inhibiting agents intervening in the various phases of vernalization, rather than to assays involving known, metabolically active substances. The exchange of material between the nucleus and the cytoplasm should be taken into consideration in terms of modern physiological genetics as discussed below.

VERNALIZATION AND DORMANCY

"Dormancy" coincides with a cessation of growth (or auxesis) caused by internal factors within the organism or organ. The growth of any developing organ is arrested since the mechanisms controlling the increase in size are inhibited. Among such mechanisms is production or effectiveness of auxin. Release from dormancy is evidenced by renewed growth, and implies a resumption of previous activities rather than the introduction of new activities. During dormancy the frequency of mitosis decreases correlatively with the inhibition of growth, e.g., a twig of *Salix repens* growing under long-day conditions forms leaves and internodes; when transferred to short-day conditions the twig forms short internodes, the leaves are reduced to the condition of scales, and the terminal bud goes into dormancy; when subjected to cold, the twig recovers its former activity (38, 40).

The shortening of internodes, termed "brachyblasty," and expressed by the "rosette" configuration, is related to dormancy since it results from a shortening of internodes, but it differs from dormancy in not interfering with the formation and expansion of leaves (43). What is affected is the mechanism of cell elongation in internodes and of cell formation in between leaf bases, but the ring of cells around the apex, from which leaves originate, retains its activity and the enlargement of leaf primordia remains unretarded.

The "nonvernalized state" may be defined as the inability to form flowers and affects the potential activity of the summit of the apex and of terminal or axillary vegetative points. These retain an "anticipating meristematic" condition that is completely independent of the production or nonproduction of leaves or internodes.

Dormancy may be broken by submitting buds to treatments apt to release inhibitions. Among external stimuli, cold is the common one, but similar effects may be achieved using heat (applied as a hot bath), drying, long days, anesthesics, glycol monochlorhydrin, thiourea, gibberellin, or an exogenous auxin. Brachyblasty, when released, is normally followed by the formation of long internodes. The release can be effected by exposure to cold or long days, or by treatment with gibberellin. Other agents that break dormancy are generally ineffective. The nonvernalized state is removed by cold or gibberellin, or rarely by either long or short days. The effectiveness of cold, and less often of gibberellin, is a feature common to the three phenomena: breaking of dormancy, releasing of brachyblasty, and vernalization.

These three phenomena, however, can be differentiated in terms of the response of each to other agents.

Dormancy may not coincide with the nonvernalized state and may intervene as flowers are being differentiated in the bud, which then ceases growth and forms leaves. The breaking of dormancy in this case has nothing to do with vernalization. The example of *Salix repens* is most illuminating (38, 47). Axillary catkins form following photosynthetic activity in the leaves. Transfer from long to short days may then be arranged to induce dormancy either before or after the catkins have differentiated. Thus, breaking dormancy may not be required for differentiation of catkins, and may therefore be confounded with vernalization or sharply separated from it. These alternatives clearly distinguish the breaking of dormancy from vernalization.

Likewise, dormancy in the seeds of *Geum* may be broken by low temperatures or by excising the integuments; neither of these treatments causes vernalization of the seeds.

Brachyblasty is closely related to the nonvernalized state, although not always. Most plants requiring vernalization are of the rosette type. Cold has a dual effect: elongation of internodes, and vernalization. Thus, vernalization can be confused with release of the inhibition of internode elongation. However, although they may be concomitant, the two phenomena are distinct, as shown by the following comparisons.

(*a*) Many plants that require vernalization are caulescent and never have a rosette phase; their vernalization is not related to the length of internodes [*Euphorbia lathyris, Teucrium scorodonia* (43, 47)].

(*b*) Gibberellin may shift the brachyblastic form to the caulescent type, without causing any vernalization [*Oenothera biennis* (201), *Scrophularia alata* (148)].

(*c*) Long days sometimes cause elongation of internodes from the rosette stage without effecting vernalization [*Salvia silvestris* (47)].

(*d*) Flowering does not necessarily depend on the elongation of the flower-bearing stem; thus, *Lavauxia* (*Oenothera*) *acaulis* develops flowers while it is a rosette, as does *Rudbeckia bicolor* (with high temperatures and short days) (226).

(*e*) Initiation of flowers may occur deep in the rosette with the flower stalk elongating later (*Spinacia oleracea*) (176), or the converse may occur [*Oenothera* sp., some strains of *Scabiosa ukranica* (47)].

(*f*) In henbane, cold-induced vernalization first entails inflorescence initiation and then elongation, whereas the situation is reversed when the inducing agent is gibberellin (228).

(*g*) In peach trees, seed dormancy is partially released with time if cold does not intervene, but the seedling can form only a rosette. Elongation is made possible following chilling or treatment with gibberellin (10). Later, in due time, flowering occurs and such a release of brachyblasty has been confused with vernalization. Flowering requires a proper equilibrium

of mild temperature and nutrition such as can be obtained with properly developed leaves. Then flowering may occur either on long twigs or on rosette-like twigs ("bouquets de Mai"). Whenever a nonvernalized state is concomitant with brachyblasty or the rosette stage, it may be said that vernalization requires at least as much, and frequently more, cold (or gibberellin) as would be required to release brachyblasty.

Vernalization and brachyblasty have common features but must be considered as different phenomena unless we recognize various types or stages of vernalization. One such stage would be completely separate from the release of brachyblasty while the others might be strictly associated with its release. One or several stages would be needed according to the species, varieties, or even genotypes. Further research is needed to solve this problem.

ELUCIDATING THE INTIMATE PROCESS OF VERNALIZATION

Vernalization fundamentally implies: (*a*) an aftereffect resulting in active mitoses spreading to the very apex of the vegetative point, which then develops into the initial inflorescence or flower; and (*b*) irreversible, or reversible, autocatalytic preservation of the after effect.

Self-perpetuating new properties are expressed either through particulate release of DNA from the nucleus—to induce autocatalytic multiplication of cytoplasmic RNA particles as carriers of the new property—or through displacements of an equilibrium involving several pre-existing and self-perpetuating enzymes (68).

Cooling may control such a displacement of equilibrium while it slows down the rate of some enzymatic reactions and speeds up others; thereby triggering a cellular reaction which becomes autocatalytic. Cooling may even directly affect nuclear properties at the time it confers the capacity for the increased rate of mitoses. Gibberellin is endowed with properties similar in many respects to those of cold. It directly controls mitosis in primary meristems. This control is independent of that exerted on enlargement via auxins (216). Similar vernalizing properties of chilling and sometimes of gibberellin may be linked with similar properties affecting nuclear activity.

Assuming that flowering is induced by some interaction of nuclear DNA with an autocatalytic cytoplasmic system, flowering regulation would then be investigated either as a release of nuclear particles, or as an inhibition of stimulation of the proliferation of such particles once they had been released into the cytoplasm, or in the stimulation of inhibition of the activity of their products. Such effects might be, in some cases, mediated via mobile and diffusible hormonal compounds. Hereditary properties would be expected to intervene in forming stable or labile linkages between active particles and nuclei. The preceding discussion features vernalization as a self-perpetuating process involving certain "cellular lines" and focuses attention on some nucleo-cytoplasmic interrelationships.

Heretofore, biochemical investigations have concentrated on the consequences rather than the causes of vernalization. New lines of research are now possible on nucleo-cytoplasmic relations based on information from the ecophysiological studies reviewed here. The available information permits a screening to select the material most likely to yield valuable information on the fundamental problems of organogenesis.

(See the Addendum following Literature Cited section.)

LITERATURE CITED

1. Aach, H. G., *Ber. deut. botan. Ges.*, **69**, 20 (1956)
2. Aach, H. G., and Melchers, G., *Biol. Zentr.*, **76**, 466–76 (1957)
3. Abolina, G., *Compt. rend. acad. sci. U.R.S.S.*, **18**, 119–202 (1958)
4. Anagnostopoulos, P. T., *Meeting Biol. Soc. Athens, Greece* (1956); *Fruits et Primeurs*, **26**, 205–17 (1956)
5. Arthur, J. M., and Harvill, E. K., *Contribs. Boyce Thompson Inst.*, **12**, 111–18 (1941)
6. Avakjan, A. A., *Agrobiologiya*, **2**, 12–21 (1950)
7. Avakjan, A. A., and Pastreb, M. G., *Agrobiologiya*, **6**, 41 (1949)
8. Avakjan, A. A., and Tagizade, N. H., *Jarovizacia*, **1**, 65 (1935)
9. Bakhuyzen, H. L. van de Sande, *Landbouwk. Tijdschr.*, **55**, 533–48 (1943); *Verslag Landbouwk. Ondirzaek.*, **53**, 4B, 145–212 (1947)
10. Barton, L. V., *Contribs. Boyce Thompson Inst.*, **27**, 311–17 (1955)
11. Bassarskaja, M. A., *Semenovodotvo*, **3**, 15–20 (1934); *Priroda*, **2**, 43–96 (1934); *Bull. Jarovizacia*, **6**, 101–8 (1936)
12. Bell, G. D. H., *J. Agr. Sci.*, **25**, 245–57 (1935); **26**, 155–71 (1936); **36**, 167–83 (1946)
13. Bendix, S., and Went, F. W., *Botan Gaz.*, **117**, 326–35 (1956)
14. Blake, J., *Bull. Soc. franc. physiol. végétale*, **2**, 169–71 (1956)
15. Blaney, L. T., *Oregon Orn. Nurs. Dig.*, **1**, 5 (1957)
16. Blaauw, A. H., *Proc. Acad. Sci. Amsterdam*, **44**, 224–52, 361–68 (1944)
17. Bonde, E. K., and More, T. C., *Physiol. Plantarum*, **11**, 451–56 (1958)
18. Calvert, A., *Ann. Rept. John Innes Hort. Inst.*, **1955**, 24–5 (1956)
19. Calvert, A., *J. Hort. Sci.*, **32**, 9–17 (1957)
20. Campbell, C. W., and Leopold, A. C., *Plant Physiol.*, **30**, 21 (1955)
21. Carr, D. J., McComb, A. J., and Osborne, L. D., *Naturwissenschaften*, **44**, 428 (1957)
22. Cathey, H. M., *Proc. Am. Soc. Hort. Sci.*, **64**, 423–91, 492–98 (1954); **68**, 386–91 (1955); **69**, 485–91 (1957).
23. Cepikova, A., *Semskosyi Pastibisca*, **1**, 432–63 (1955)*
24. Cesnokov, V., *Trudy Leningrad Obshchestva Estestvoispytatelei Bot.*, **63**, 110–12 (1934)
25. Chakravarti, S. C., *Indian J. Agr. Sci.*, **23**, 289–300 (1953)
26. Chakravarti, S. C., *Nature*, **182**, 1612–13 (1958)
27. Chakravarti, S. C., and Pillai, I. N. L., *Phyton*, **5**, 1–17 (1955)
28. Champéroux, A., *Thèse* 3ème Cycle, Paris, France (In press)
29. Chan, A. P., *Rept. 14th Intern. Hort. Congr.*, Netherlands, 1023–39 (1955)
30. Chestakov, V. E., and Sergeev, L. I., *Comp. rend. acad. sci. U.R.S.S.*, **4**, 1 (1937)
31. Chinoy, J. J., *Physiol. Plantarum*, **9**, 1–18 (1956)
32. Cholodny, N. G., *Compt. rend. acad. sci. U.R.S.S.*, **3**(8), 391–94 (1936)
33. Chouard, P., *Compt. rend. acad. agr. France*, **24**, 312–17 (1938); *Compt. rend. soc. biol.*, **127**, 66–7 (1938); *Le Chrysanthème*, 4 pp. (1946)
34. Chouard, P., *Bull. soc. botan. France*, **96**, 218–20, 235–38 (1949)
35. Chouard, P., *Bull. soc. botan. France (Mémoires 1949)*, 106–46 (1949)
36. Chouard, P., *Pourquoi fleurissent les plantes?* (Conf. Palais de la Découverte, Paris, France, 62 pp., 1949)

37. Chouard, P., *Compt. rend.*, **233,** 86–88 (1951); *Bull. soc. botan France,* **98,** 273–76 (1951)
38. Chouard, P., *Bull. soc. botan. France (Mémoires 1950/51),* 67–81 (1950)
39. Chouard, P., *Bull. soc. botan. France,* **98,** 11–20 (1951)
40. Chouard, P., *Dormances et inhibitions des graines et des bourgeons; préparation au forçage; thermopériodisme* (S.E.D.E.S., Paris, France, 157 pp., 1951)
41. Chouard, P., *Rept. of the 14th Intern. Hort. Congr.,* Netherlands, 14–38 (1955)
42. Chouard, P., *Bull. Soc. franc., physiol. végétale,* **2,** 125–36 (1956)
43. Chouard, P., *Bull. soc. botan. France (Mémoires 1956/57),* 52–64 (1957); *Rev. horti. (Paris),* **222,** 1792–1803 (1958)
44. Chouard, P., *Compt. rend.*, **245,** 2361–63 (1957)
45. Chouard, P., *Compt. rend.*, **245,** 2520–22 (1957)
46. Chouard, P., *Bull. soc. botan. France,* **105,** 135–36 (1958)
47. Chouard, P., *Colloq. intern. Photo-thermopériodisme, Parma, Italy, 1957,* 7–23 (1959)
48. Chouard, P., and Poignant, P., *Compt. rend.,* **232,** 103–105 (1951); *Phytoma,* **1,** 120–22 (1951)
49. Chouard, P., and Vaidie, P., *Compt. rend.,* **207,** 1444–46 (1938)
50. Chouard, P., and Weber, M. R., *Compt. rend.,* **243,** 1659–61 (1956)
51. Chroboczek, E., *Mem. Cornell Univ. Agr. Expt. Sta.,* **154,** 84 pp. (1934); and Chroboczek, E., and Kosteckii, T. *Ann. Sci. Hort.,* **4,** 153–75 (1937)
52. Chroboczek, E., *Rept. 14th Intern. Hort. Congr.* Netherlands, 429–39 (1955)
53. Cooper, J. P., *J. Ecol.,* **39,** 228–70 (1951)
54. Curtis, D. S., and Chan, H. T., *Am. J. Botany,* **17,** 1047–48 (1930)
55. Czailachjan, M. C., *J. Botan. Moscou,* **41,** 487–509 (1956)
56. Czailachjan, M. C., and Kuznetzova, I. C., *Compt. rend. acad. sci. U.R.S.S.,* **105,** 842–45 (1955)
57. Czailachjan, M. C., and Zdanova, L., *Bull. acad. sci. U.R.S.S., Ser. biol.,* **2,** 523–38 (1938)
58. Dao-The-Touan, *Agrobiologiya,* **115,** 79–85 (1959)*
59. David, R., *Bull. muséum hist. nat. Marseille,* No. 4, 242 (1942)
60. David, R., *Rev. sci.,* 508–10 (1943); 106–8, 309–10, 459–60 (1944); 46–48 (1945)
61. David, R., *Rev. Sci.,* 374–75 (1944)
62. David, R., *Compt. rend. soc. biol.,* **138,** 849–51 (1944); **139,** 560–62 (1945)
63. David, R., *Facteurs de développement et printanisation des végétaux cultivés* (Act. Sc. et Int., No. 1013, Hermann, et Cie., Paris, France, 177 pp., 1946)
64. David, R., *Compt. rend.,* **224,** 146–47 (1947); **233,** 428–30 (1951)
65. David, R., and Séchet, J., *Compt. rend. soc. biol.,* **141,** 459 (1947)
66. David, R., and Séchet, J., *Compt. rend.,* **227,** 537–38 (1948)
67. David, R., and Séchet, J., *Rev. Sci.,* 542–44 (1948)
68. Delbrück, M., in: *Unités biologiques douées de continuité génétique,* pp. 33–35 [Colloq. intern. centre nat. rechenche Sci. (Paris), 1949]
69. Demkovskii, P. I., *Bull. Jarov.,* **1,** 42–46 (1932)
70. Denffer, D. von, *Jahrb. wiss. Botan.,* **88,** 759–813 (1939)
71. Dikshit, N. N., and Singh, U. P., *Current Sci. (India)* **21,** 249–50 (1952)
72. Doorenbos, J., *Mededeel. Dir. Tuinb.,* **18,** 375–90 (1955); *Bull Soc. franc. physiol. végétale,* **2,** 150–61 (1956); *Colloq. Intern. Photo-thermopériodisme, Parma, Italy, 1957,* 51–54 (1959)

73. Dupéron, R., *Rev. gén. botan.*, **53,** 525–57 (1946)
74. Dupéron, R., *Compt. rend.*, **228,** 192–94 (1949); **233,** 1053–55 (1951)
75. Dupéron, R., *Influence de la vernalisation sur le métabolisme,* (Thèse, Paris France, 131 pp. 1951) and *Rev. gén. Botan.*, **59,** 580–631 (1952); **60,** 33–78, 90–122 (1953)
76. Dupéron, R., *Bull. Soc. franç. physiol. végétale,* **2,** 144–52 (1956)
77. Efeikin, A. K., *Compt. rend, acad. sci. U.R.S.S.*, **25,** 308–10 (1939); **30,** 661–63 (1941); **56,** 95–97 (1947)
78. Eguchi, T., *et al., Wogyô Gijutsu Kenkyûja Hokoku, E,* **3,** 217–64 (1955); (Anal. in *Hort. Abstr.*, No. 424, 1957)
79. Emswiller, S. L., and Borthwick, H. A., *Proc. Am. Soc. Hort. Sci.*, **33,** 755–57 (1937)
80. Eremenko, V. T., *Sovet. Botan.*, **6,** 36–45 (1955)
81. Feodorov, A. K., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **5,** 62–69 (1958)
82. Feofanova, N. D., *Compt. rend. acad. sci. U.R.S.S.*, **68,** 181–94 (1949)
83. Filippenko, I. A., *Compt. rend. acad. sci. U.R.S.S.*, **3,** 185–89 (1936)
84. Filippenko, I. A., *Compt. rend. acad. sci. U.R.S.S.*, **28,** 167 (1940)
85. Friedberg, M., *Compt. rend. acad., agr. France,* **20,** 220–28 (1934)
86. Friend, D. J., and Gregory, F. G., *Nature,* **172,** 667–68 (1953)
87. Fukui, H. N., Weller, L. E., Wittwer, S. H., and Sell, H. M., *Am. J. Botany,* **45,** 73–4 (1958)
88. Gardner, F. P., and Loomis, W. E., *Plant Physiol.*, **28,** 201–17 (1953)
89. Gassner, G., *Z. Botan.*, **10,** 419–30 (1918)
90. Gassner, G., *Züchter,* **23,** 193–206 (1953)
91. Glover, J., *Ann. Botany (London),* **19,** 138–48 (1955)
92. Goebel, Christa, *Coll. Techn. Tübingen, Thesis (1950); Z. Naturforsch.* **56,** 49–50 (1950)
93. Goodall, D. W., and Bolas, B., *Ann. Appl. Bot.*, **29,** 1–10 (1942)
94. Gott, M. B., Gregory, F. G., and Purvis, O. N., *Ann. Botany (London), 21,* 87–126 (1955)
95. Gravilova, M. F., *Compt. rend. acad. sci. U.R.S.S.*, **7–8,** 561–63 (1935)
96. Gray, S. G., *J. Council Sci. Ind. Research* **15,** 3 (1942)
97. Gregory, F. G., *Nature,* **138,** 249, 923 (1936)
98. Gregory, F. G., *Symposia Soc. Exptl. Biol. No. 2,* 75–103 (1948)
99. Gregory, F. G., and De Ropp, R. S., *Nature,* **142,** 481–88 (1938)
100. Gregory, F. G., and Purvis, O. N., *Nature,* **138,** 973 (1936)
101. Gregory, F. G., and Purvis, O. N., *Nature,* **139,** 1013–14 (1936)
102. Gregory, F. G., and Purvis, O. N., *Nature,* **140,** 547–48 (1937)
103. Gregory, F.G., and Purvis, O. N., *Ann. Botany (London)* **2,** 237–51 (1938)
104. Gregory, F. G., and Purvis, O. N., *Ann. Botany (London),* **2,** 753–64 (1938)
105. Gregory, F. G., and Purvis, O. N., *Nature,* **161,** 859–61 (1948)
106. Günther, G., *Ber. deut. botan. Ges.*, **66,** (20)–(21) (1955)
107. Hänsel, H., *Ber. Arbeitstag* 1952, *Arbeitsgemeinsch. Staatzuchtleiter Admont.*, 66–120 (1952)
108. Hänsel, H., *Ann. Botany (London),* **17,** 417–32 (1953)
109. Harrington, J. F., Rappaport, C., and Hood, K. J., *Science,* **125,** 601–2 (1957)
110. Hartmann, H. T., and Polinga, I., *Botan. Gaz.*, **119,** 102–4 (1957); *Calif. Agr.*, **12,** 6–11 (1958)

111. Hartmann, T. A., *Koninkl. Ned. Akad. Wetenschap., Proc., Ser. C*, **59**, 677–86 (1956)
112. Hatcher, E. S. J., *Nature*, **151**, 278 (1943); *Ann. Botany (London)*, **9**, 235–66 (1945)
113. Hatcher, E. S. J. and Purvis, O. N., *J. Agr. Research*, **35**, 117–83 (1945)
114. Haupt, W., *Z. Botan.*, **40**, 1–32 (1952); **46**, 242–56 (1958); *Ber. deut. botan. Ges.*, **62**, 71–88 (1954)
115. Henninger, J., *Dipl. ét. sup., Sorbonne*, Paris, (May 1959)
116. Highkin, H. R., *Plant Physiol.* **30**, xxi (1955)
117. Highkin, H. R., *Plant Physiol.* **31**, 399–403 (1956)
118. Highkin, H. R., quoted by F. Went in, *Bull. Soc. franc., physiol. végétale*, **2**, 156 (1956)
119. Highkin, H. R., *Am. J. Botany*, **45**, 626–31 (1958)
120. Hussey, G., and Gregory, F. G., *Plant Physiol.*, **29**, 292–296 (1954)
121. Inoue, Y., and Suzuki, E., *J. Hort. Assoc. (Japan)*, **23**, 177–82 (1954)
122. Ivanov, S. M., *Trudy Priklad. Botan. Genet. i Selektsii*, 33–39 (1934)
123. Jouanneau, P., *Dipl. Et. Sup., Sorbonne*, Paris (Nov. 1958)
124. Junges, W., *Archiv. Gartenbau*, **2**, 213–24 (1954)
125. Junges, W., *Archiv. Gartenbau*, **3**, 297–315 (1955)
126. Junges, W., *Ber deut. botan. Ges.*, **71**, 197–204 (1958)
127. Kagawa, A., *J. Hort. Assoc. (Japan)*, **25**, 173–80 (1956)
128. Klippart, J. H., *Ann. Rept. Ohio State, Bd. Agr.*, Sect. 12, 562–816 (1857)
129. Kloen, D., *Congr. intern. botan., 8e Congr., Paris*, **11**, 291-92 (1954)
130. Konovalov. I. N., *Soc. Zern. Hoz.*, **1**, 9 (1934); (Quoted by Séchet, J., 1953)
131. Kostjucenko, I. A., and Zarubailo, T. F., *Selekcija i Semenov.*, No. 3, 49 (1935); *Compt. rend. acad. sci. U.R.S.S.*, **18/19**, 84 (1938)
132. Krasnoseljskaja-Maximova, T. A., *Trudy Priklad. Botan. Genet. i Selektsii*, **27**, 113–28 (1931)
133. Kruzilin, A. S., and Svedskaja, Z. M., *Compt. rend. acad. sci. U.R.S.S.*, **98**, 487–90 (1954)
134. Kruzilin, A. S., and Svedskaja, Z. M., *Compt. rend. acad. sci. U.R.S.S.*, **116**, 870–73 (1957)
135. Kruzilin, A. S., and Svedskaja, Z.M., *Agrobiologiya*, No. 3, 118–22 (1957); *Vestnik Sci. Agr. Moscow*, No. 12, 46–52 (1958)*
136. Kugler, I., *Beitr. Biol. Pflanz.*, **28**, 21–43 (1951)
137. Kumaki, Y., *J. Hort. Assoc. (Japan)*, **25**, 163–66 (1956)
138. Laibach, F., *Beitr. Biol. Pflanz.*, **28**, 173–210 (1951)
139. Lang, A., *Fortschr. Botan.*, **11**, 340–441 (1949)
140. Lang, A., *Ann. Rev. Plant Physiol.*, **3**, 265–306 (1952)
141. Lang, A., *Fortschr. Botan.*, **15**, 400–75 (1954); **16**, 342–376 (1954)
142. Lang, A., *Plant Physiol.*, **31**, xxv (1956); *Naturwissenschaften*, **43**, 284 (1956)
143. Lang, A., *Proc. Natl. Acad. Sci. U.S.*, **43**, 709–17 (1957); *Colloq. intern. Photothermopériodisme, Parma, Italy, 1957*, 55–74 (1959)
144. Lang, A., and Melchers, G., *Planta*, **33**, 653–702 (1943); *Z. Naturforsch.*, **2b**, 444–49 (1947)
145. Lang, A., and Melchers, G., *Z. Naturforsch.*, **3b**, 208 (1948)
146. Lang, A., Sandoval, J. A., and Bechi, A., *Proc. Natl. Acad. Sci. U.S.*, **43**, 960–64 (1957)

147. Larose, E., and Vanderwalle, R., *Bull. Inst. agron. et stat. recherches Gembloux*, **5**, 160–80 (1935)
148. Larrieu, C., *Dipl. Et. Sup.*, Sorbonne, Paris, 1959–60 (In press)
149. Lé, K. N., *Thesis 3éme Cycle*, Sorbonne, Paris, 1959–60 (In press)
150. Leopold, A. C., and Guernsey, F. S., *Am. J. Botany*, **41**, 181–85 (1954)
151. Lerman, R. I., *Izvestia Int. Sc. nat. Lesgaft*, **27**, 135–47 (1955)
152. Lojkin, M., *Contribs. Boyce Thompson Inst.*, **8**, 237–61 (1936)
153. Lona, F., *Nuova giorn. botan. ital*, **63**, 61–76 (1956); *Colloq. intern. Photothermopériodisme, Parma, Italy, 1957*, 41–68 (1959)
154. Lona, F., and Bocchi, A., *Riv. intern. Agra.*, No. 6, 44 (1956)
155. Lysenko, T. D., *Trudy Azerbaidj., Op. St.*, **3**, 1–168 (1928)
156. Lysenko, T. D., *Bull. Jarovizacii*, **4**, 1–57 (1932)
157. Lysenko, T. D., *Theoretical Basis of Vernalization* (Moscow, 1935)
158. Lysenko, T. D., *Agrobiologiya, Selfcozgiz* (1952) (quoted by Razumov, 1954)
159. Lysenko, T. D., *Stadiinoe razvitie rastenii., Selicozgiz* (1952) (quoted by Razumov, 1954)
160. McKee, R., *U.S. Dept. Agr. Circ.*, **377**, 12 pp. (1935)
161. McKinney, H. H., and Sando, W. J., *J. Heredity*, **24**, 169–79 (1933)
162. McKinney, H. H., and Sando, W. J., *J. Agr. Research*, **51**, 621–41 (1935)
163. Margadant, W. D., *Verslag. Landbouwk. Onderzoek., B* (1951) (quoted by Wycherley, P. R., 1952)
164. Margara, J., *Compt. rend.*, **246**, 145–47 (1958); *Compt. rend.*, (Aug. 1959; In press)
165. Maximov, N. A., *Biol. Zentr.*, **49**, 513–43 (1929)
166. Maximov, N. A., *Bull. Imp. Bur. Pastures Forage Crops*, No. 16, 5–14 (1934)
167. Maximov, N. A., and Pojarkova, A. T., *Jahrb. wiss. Botan.*, **64**, 702–30 (1925)
168. Melchers, G., *Biol. Zentr.*, **56**, 567–570 (1936)
169. Melchers, G., *Biol. Zentr.*, **57**, 568–614 (1937)
170. Melchers, G., *Biol. Zentr.*, **57**, 643 (1937)
171. Melchers, G., *Naturwissenschaften*, **26**, 496 (1938)
172. Melchers, G., *Ber. deut. botan. Ges.*, **57**, 29–48 (1939)
173. Melchers, G., *Rend. ist. lombardo sci.*, **83**, 1–28 (1950)
174. Melchers, G., *Lect. mimeogr. Res. Inst. Plant Physiol.*, Imp. Coll. London, 1–33 (1952)
175. Melchers, G., und Claes, H., *Naturwissenschaften*, **31**, 249 (1943)
176. Melchers, G., und Lang, A., *Biol. Zentr.*, **61**, 16–39 (1941)
177. Melchers, G., und Lang, A., *Biol. Zentr.*, **67**, 105–174 (1948)
178. Melchers, G., und Lang, A., *Z. Naturforsch.*, **3b**, 105–7 (1948)
179. Met, M., *Rev. gén. botan.*, **57**, 429–451 (1950)
180. Miller, J. C., *Cornell Univ. Agr. Expt. Sta.*, Bull. No. 488 (1929)
181. More, T. C., and Bonde, E. K., *Physiol. Plantarum*, **11**, 752 (1958)
182. Munerati, O., *Giorn. Agr. Domen.*, **44**, 245 (1934)
183. Murneek, A. E., and Whyte, R. D., *Vernalization and Photoperiodism* (Lotsya, A., Ed., Chronica Botanica, Waltham, Mass., 193 pp., 1948)
184. Napp-Zinn, K., *Ber. deut. botan. Ges.*, **66**, 362–67 (1953); *Congr. intern. botan. 8th Congr., Paris, 1954*, Sect. 11, 288–90 (1954); *Flora*, **144**, 402–18 1956–57)
185a. Napp-Zinn, K., *Z. Botan.*, **45**, 379–96 (1957)
185b. Napp-Zinn, K., *Z. Botan.*, **46**, 506–15 (1958)

186a. Napp-Zinn, K., *Planta,* **50,** 177–210 (1957)
186b. Napp-Zinn, K., *Planta,* **52,** 229–34 (1958)
187. Nakamura, E., *J. Hort. Assoc. (Japan),* **25,** 167–72 (1956)
188. Newell, L. C., *Agron. J.,* **43,** 417–24 (1951)
189. Noggle, G. R., *Plant Physiol.,* **21,** 492–505 (1946)
190. Nutman, P. S., *Ann. Botany (London),* **5,** 353–74 (1941)
191. Ovetchkine, C. K., *Comp. rend. acad. sci. U.R.S.S.,* **74,** 147–48 (1950)
192. Owen, F. V., Carsner, E., and Stout, M., *J. Agr. Research,* **61,** 101–24 (1940)
193. Paci, P., *Riv. ortoflorofrutticult. ital.,* **40,** 320–331 (1956)
194. Pal, B. P., and Marty, S. G., *Indian genet. pl. Breed.,* **1,** 61–68 (1941)
195. Palmer, J. H., *The New Phytologist,* **57,** 145–59 (1959)
196. Papadakis, J. S., *Ecologie Agricole,* J. Duculot, Gembloux (1938)
197. Paton, D. M., *Plant Physiol.,* **32,** ix–x (1957)
198. Paton, D. M., and Barber H. M., *Australian. J. Biol. Sci.,* **8,** 231–40 (1955)
199. Peterson, M. L., and Loomis, W. E., *Plant Physiol.,* **24,** 31–43 (1949)
200. Peto, F. H., *Can. J. Sci.,* **2**(6) (1934) (quoted by Stokes, P., and Verkert, K., 1951) 2413 1665-68
201. Picard, C., *Compt. rend.,* **244,** 1685–88 (1956); **247,** 2186–87 (1958); *Compt. rend.,* Aug. 1959 (In press)
202. Pojarkova, S. J., *Ber. deut. botan. Ges.,* **45,** 627–37 (1927)
203. Post, K., *Proc. Am. Soc. Hort. Sci.,* **33,** 649–52 (1936); *Rept. Intern. Hort. Congr., 13th Congr., London, 1952,* 935–40 (1952)
204. Poux, N., *Rev. cytol. et biol. végétales,* **19,** 273–310 (1958)
205. Povar, M. P., *Agrobiologiya,* **115,** 141 (1959)*
206. Purvis, O. N., *Ann. Botany (London),* **48,** 419–95 (1934)
207. Purvis, O. N., *Ann. Botany (London),* **31,** 219–31 (1939); *Nature,* **145,** 462 (1940)
208. Purvis, O. N., *Ann. Botany (London),* **8,** 285–314 (1944)
209. Purvis, O. N., *Ann. Botany (London),* **11,** 269–83 (1947)
210. Purvis, O. N., *Ann. Botany (London),* **12,** 183–206 (1948)
211. Purvis, O. N., *Congr. intern. botan., 8e Congr., Paris,* Sect. 11, 286–88 (1954)
212. Purvis. O. N., and Gregory, F. G., *Ann. Botany (London),* **1,** 569–92 (1937)
213. Purvis, O. N., and Gregory, F. G., *Nature,* **155,** 163 (1945)
214. Purvis, O. N., and Gregory, F. G., *Ann. Botany (London),* **16,** 1–21 (1952)
215. Purvis, O. N., and Gregory, F. G., *Nature,* **171,** 687–88 (1953)
216. Rappaport, L., and Wittwer, S. H., *Plant Physiol.* **30,** xx–xxii (1955); *Proc. Am. Soc. Hort. Sci.,* **67,** 429–37 (1956); **67,** 438–9 (1956)
217. Rappaport, L., Wittwer, S. H., and Tuckey, H. N., *Nature,* **178,** 51 (1956)
218. Rasumov, V. I., *Agrobiologiya,* **2,** 27–24 (1950)
219. Rasumov, V. I., *Sreda i osobennosti razvitia rastenii,* Moscou and Leningrad, 143 pp. (1954)
220. Rasumov, V. I., Feokanova, N. D., and Oleinikova, T. V., *Compt. rend. acad. sci. U.R.S.S.,* **60,** 689–95 (1948)
221. Rasumov, V. I., Oleinikova, T. V., and Jordanov, I. T., *Fiziol. Rastenii, Akad. Nauk, S.S.S.R.,* **1,** 73–80 (1954)
222. Rasumov, V. I., and Oleinikova, T. V., *Fiziol. Rastenii, Akad. Nauk, S.S.S.R.,* **5,** 497–503 (1955)
223. Rasumov, V. I., and Smirnova, M., *Compt. rend. acad. sci. U.R.S.S.,* **60,** 917–19 (1948)

224. Richter, A. A., *Priroda*, **2**, 43–46 (1934)
225. Richter, A. A., Rancan, V. A., and Pekker, M. Z., *Compt. rend. acad. sci. U.R.S.S.*, **2**, 72–77 (1933)
226. Roberts, R. H., and Struckmeyer, B. E., *J. Agr. Research*, **56**, 633–78 (1938); **59**, 699–709 (1939)
227. Sapoznikova, K. K., *Trudy biol. Inst. Tomsk. Univ.*, **1**, 238 (1935)
228. Sarcar, S., *Biol. Zentr.*, **77**, 1–49 (1958)
229. Schmalz, H., *Z. Pflanzenzucht.*, **147**, 38 pp. (1957)
230. Schwabe, W. W., *J. Exptl. Botany*, **1**, 329–43 (1950)
231. Schwabe, W. W., *J. Exptl. Botany*, **2**, 223–37 (1951)
232. Schwabe, W. W., *J. Exptl. Botany*, **3**, 230–35 (1952); **6**, 435–50 (1955)
233. Schwabe, W. W., *J. Exptl. Botany*, **5**, 389–400 (1954)
234. Schwabe, W. W., *Nature*, **174**, 1022 (1954)
235. Schwabe, W. W., *J. Exptl. Botany*, **8**, 220–34 (1957)
236. Schwabe, W. W., *Symposium on Control of the Plant Environment; Nottingham, England*, 16–33 (1957)
237. Seelhorst, C., von., *J. Landwirtsch.*, **46**, 50 (1898) (quoted by David, R., 1946, and Séchet, J., 1953)
238. Sen, B., and Chakravarti, S. C., *Indian J. Agr. Sci.*, **3**, 245–52 (1938); *Nature*, **157**, 266 (1946); **159**, 783 (1947)
239. Séchet, J., *Contribution à l'étude de la printanisation* (Thèse Bordeaux, 289 pp., 1953; *Le Botaniste*, **37**, 1–289 (1953)
240. Séchet, J., *Compt. rend. acad. agr. France*, **39**, 265–67 (1953)
241. Séchet, J., *Bull. soc. franç. physiol. végétale*, **2**, 163–64 (1956)
242. Sereiskii, A., and Sluckaja, M., *Botan. Zhur.*, **19**, 311–29 (1954)*
243. Simpson, A. C., *Nature*, **151**, 279–80 (1943)
244. Sircar, S. M., 121–8, in *Vernalization and Photoperiodism* by Murneek, A. E., and Whyte, R. D., (Chronica Botanica, Waltham, Mass., 193 pp., 1948)
245. Sircar, S. M., and Parya, B., *Nature*, **155**, 395 (1945)
246. Sisakyan, N. M., *Sessiya extr. Akad. Lénine Sci. Agr. U.R.S.S., 1948* (Moscow, 1949) (quoted by Séchet, J., 1953, and Dupéron, R., 1956)
247. Sisakyan, N. M., and Filippovich, I. I., *Zhur. Obshchei Biol.*, **14**, 215 (1953)
248. Skripchibokii, V. V., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **5**, 166–74 (1958)
249. Stokes, P., and Verkerk, K., *Mededel. Landbouwhogeschool Wageningen*, **50**, (9), 141–60 (1951)
250. Stout, M., *Botan. Gaz.*, **72**, 49 (1945); *J. Agr. Research*, **72**, 49 (1946)
251. Stout, M., *Botan. Gaz.*, **107**, 86 (1950)
252. Stroun, M., *Contribution à l etude du developement des Céréales* (Thesis, Geneva, 1957; Lechevalier, P., Ed., Encyclopedie Biologique, Paris, France, 192 pp., 1958)
253. Suin, K. A., *Sad i Ogorod*, No. 2, 34–35 (1955)
254. Sutov, D. A., and Beljaev, N. V., *Izv. Moldavsk. Filiala Akad. Nauk S.S.S.R.*, No. 6, 101–16 (1955)
255. Tetjurev, V. A., *Compt. rend. acad. sci. U.R.S.S.*, **25**, 627 (1939); **30**, 661–63 (1941)
256. Thompson, H. C., *Proc. Am. Soc. Hort. Sci.*, **45**, 425–430 (1954)
257. Thompson, H. C., 137–46 in *Growth and Differentiation in Plants* (Loomis, W. E., Ed., Ames, Iowa, 1946)

258. Tolmacev, I. *Trudy Vsesoyuz. Sézda. Génét.*, **3**, 539 (1927)
259. Tumanov, I. I., and Trounova, T. I., *Fiziol. Rostenii, Akad. Nauk S.S.S.R.*, **5**, 112–22 (1958)
260. Verkerk, K., *Proc. Acad. Sci. Amsterdam, Ser. C*, **57**, 339–346 (1954)
261. Verkerk, K., *Mededel. Landbouwhogeschool Wageningen*, **55**, 175–224 (1955)
262. Vince, D., *J. Hort. Sci.*, **30**, 34–42 (1955); *Bull. soc. franc. physiol. végétale.*, **2**, 165–69 (1956)
263. Vince, D., and Mason, D. T., *Nature*, **174**, 842 (1954); *J. Hort. Sci.*, **32**, 184–194 (1957)
264. Vlitos, A. J., and Meudt, W., *Contribs. Boyce Thompson Inst.*, **18**, 159–66 (1955)
265. Voronova, A. E., *Sad i Ogorod*, No. 12, 19–27 (1956)
266. Voss, J., *Pflanzenbau*, **15**, 1–35, 49–79 (1938)
267. Warne, L. G. G., *Nature*, **159**, 31–32 (1947)
268. Waterschoot, H. F., *Proc. Acad. Sci. Amsterdam, Ser. C*, **60**, 318–323 (1957)
269. Weber, M. L., *Bull. soc. franç. physiol. végétale*, **2**, 164–65 (1956)
270. Wellensiek, S. J., *Vakblad. v. d. Bloemistry*, **4**, 235 (1949); **6**, 139 (1951)
271. Wellensiek, S. J., *Meded. dir. v. d. Tuinb.*, **15**, 499–521 (1952)
272. Wellensiek, S. J., *Proc. Acad. Sci. Amsterdam, Ser. C*, **62**, 115–18 (1953)
273. Wellensiek, S. J., *Rappt. général Cong. Hort. Nice, France (1958)* (In press)
274. Wellensiek, S. J., Doorenbos, J., and Zeevaart, J. A. D., *Bull. soc. franç. physiol. végétale*, **2**, 136–144 (1956)
275. Wellensiek, S. J., and Hakkaart, F. A., *Proc. Acad. Sci. Amsterdam, Ser. C*, **58**, 16–21 (1955)
276. Wellensiek, S. J., and Verkerk, K., *Neth. J. Agr Sci.*, **2**, 78–104 (1954)
277. Wellensiek, S. J., Waterschoot, H. T., and Doorenbos, J., *Handel, 32e Ned. Nat. Geneesk. Congr.*, 88–91 (1951)
278. Went, F. W., (cf. 115–26), *The Experimental Control of Plant Growth* (Chronica Botanica, Waltham, Mass., 343 pp., 1957)
279. Whyte, R. O., *Biol. Revs., Cambridge, Phil. Soc.*, **14**, 5–87 (1939); *Crop. Production and Environment* (Faber and Faber, London, England, 1947); and 1–38 in *Vernalization and Photoperiodism*, Murneek, A. E., and Whyte, R. D. (Chronica Botanica, Waltham, Mass., 193 pp., 1948)
280. Wittwer, S. H., Bukovac, M. J., Sell, H. M., and Weller, L. E., *Plant Physiol.*, **32**, 39–41 (1957)
281. Wittwer, S. H., and Teubner, F. G., *Quart. Bull. Mich. Agric. Exp. Sta.*, **36**, 198–207 (1956); *Am. Veg. Grow.*, **4**, 34–35 (1956); *Proc. Am. Soc. Hort. Sci.*, **67**, 369–76 (1956); *Am. J. Botany*, **44**, 125–29 (1957)
282. Wort, D. J., *Botan. Gaz.*, **101**, 457–80 (1939)
283. Wort, D. J., *Plant Physiol.*, **15**, 137–41 (1940)
284. Wort, D. J., *Botan. Gaz.*, **102**, 725–37 (1941)
285. Wycherley P. R., *Mededel. Landbouwhogeschool Wageningen*, **52**, 75–92 (1952)
286. Yamasaki, K., *Tokai-Kinki Nôgyô Shinkenjo Kenkyû Hôkoku No. 3*, 31–47 (1956)
287. Zan el Abdin, *Z. Botan.*, **44**, 207–20 (1956)
288. Zarubailo, I. J., *Selek. Semenovod*, **89**, 26–27 (1938)
289. Zeevaart, J. A. D., *Bull. soc. franç. physiol. végétale*, **2**, 162–63 (1956)
290. Zenker, A. M., *Beitr. Biol. Pflanz.*, **32**, 135–70 (1955)

ADDENDUM

During the 9th International Botanical Congress, held in Montreal late in August of 1959, the problem of vernalization was dealt with during several sessions. We would like to report the data emphasized there and make some comments.

In winter cereals, the vernalization effect of chilling also acts on leafy plants as long as flower initiation has not occurred spontaneously without chilling (310).

The vernalization requirement appears quite variable in different races of red clover for example (311). As regards *Oenothera,* the differences between the author's findings and those of others resulted from the fact that, under the same name, they were speaking of different plants: the genetically controlled *O. biennis,* from the Munich Botanical Gardens (309) is not the same plant as the wild *O. biennis* from the Berlin area (307). Alternating temperatures have been confirmed as very effective and precise in the true *Oenothera biennis* (309).

Vernalization is effective on germinating seed of stock (301), endive (300), and chickory (297). In endive (302), after insufficient chilling, vernalization can be attained with short days, though the quickest flowering is caused by long days given as supplementary incandescent light.

The requirements for vernalization may vary according to the bud concerned: in *Limum alpinum* (293), the main shoot requires vernalization, but axilary shoots that appear after the head is removed have either low requirements or none at all.

Vernalizability varies with plant age; in *Lunaria biennis* (313), the seed does not seem vernalizable and yet it records a chilling effect that later appears in the smaller chilling requirement of the adult plant for vernalization. In the *Oenothera* of Berlin (307), vernalizability starts at 30 days of age and shows 2 maxima, one at 56 days and the other at 180 days. In *Arabidopsis thaliana* (308), a minimum vernalizability is observed at 45 days of age under low light, and at 7 days under intense light. Illumination during the vernalizing treatment increases the effectiveness of the treatment in plants less than 50 days old. When applied in that way, vernalization is nearly wholly fixed at the end of the treatment, whereas it requires a 4-day fixation at 20°C., with light, when it has been applied in the dark.

Several plants, mostly crucifers, show the same phenomenon as recorded in Brussels sprouts: for example, in stock (301), chilling is not only a "preparatory" condition, but is also required for the realization of the process of flowering. The first flower buds can only form in the cold; then later, the others can keep on blooming in a warm temperature. Something similar occurs in *Lunaria biennis* (313) at 6-8°C.

Whereas tubers are necessary for chilling efficiency in the beet and in carrot buds, leaves are useless, but they later become necessary for further flowering in a warm temperature even if tubers are present (303).

During germination of cereal embryos, indole-3-acetic acid increases the ascorbic acid production at the expense of sucrose, more so at a low temperature than at a high one, an observation supposedly related to the chilling vernalizing effect (292). However, when vernalization occurs below 0°C. (at −2°C., for example) mitoses completely stop (299).

The action of gibberellin has often been reported, extending or confirming what has been said above, either in general (295, 304, 306), in brachyblast shoots of a half-dormant peach tree (298), in *Oenothera* (309), in *Geum* (305), or in various seeds that are vernalized by cold and not by gibberellin (291). Gibberellin is certainly not the direct cause either of flowering or of the production of flowering ability, though it may restrain flowering in certain cases, when no elongating occurs during which it would act. The various factors in vernalization may result from an inability for elongation (necessary to flowering), or from an inability for flowering, or from both (295). Czajlahjan (296) thinks gibberellin is the growth factor that is lacking under short-day conditions in long-day plants, that "anthesine" is the flowering factor that is lacking under long-day conditions in short-day plants, and that they both constitute the proper florigen. This tempting hypothesis cannot be applied to long-day caulescent plants under short-day conditions nor to caulescent biennials before chilling, in which gibberellin cannot ever cause flowering (295).

A few more suggestions were made (294 to 312) concerning the above work schedule. It was supported in its general form and it seems likely that in about 10 years, through one or another of the suggested ways, that the study of vernalization will make considerable progress.

LITERATURE CITED (SUPPLEMENT)

291. Chakravarti, S. C., *Nature*, **182**, 1612–13 (1958)
292. Chinoy, J. J., and Nanda, K. K., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 69 (1959)
293. Chodat, P., and Bang, K. D., *Bull. soc. franç. physiol. végétale*, **4**, 83–84 (1958)
294. Chouard, P., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 70–71 (1959)
295. Chouard, P., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 71 (1959)
296. Czajlahjan, M. C., *Fiziol. Rastenii Akad. Nauk, S.S.S.R.*, **5** (6), 541–60 (1958); *Biol. Zentr.*, **77**, 642–62 (1958); *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 63 (1959)
297. Doorenbos, J., and Riemens, P. C., *Acta Botan. Neerl.*, **8**, 63–67 (1959)
298. Flemion, F., *Contribs. Boyce Thompson Inst.*, **20**, 57–70 (1950); *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 114 (1959)
299. Grif., V. G., *Fiziol. Rastenii Akad. Nauk, S.S.S.R.*, **5** (6), 521–24 (1958)
300. Harrington, J. F., Verkerk, K., and Doorenbos, J., *Neth. J. Agr. Sci.*, **7**, 68–74 (1959)
301. Kohl, H. C., Jr., *Proc. Am. Soc. Hort. Sci.*, **72**, 481–84 (1958)
302. Kokin, A. J., and Členokova, I. V., *Uč. Zap Petrozavdskogo, Un-ta;* 71–82 1956; 1957); [anal. in *Hort. Abstr.*, **29** (3), 506 (No. 2745) (1959)]*
303. Kruzilin, A. C., and Svedskaja, Z. M., *Doklady Akad Nauk S.S.S.R.*, **124** (6), 453–56 (1959)
304. Lang, A., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 215 (1959)
305. Lé, Kiem Ngoc, *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II A**, 21 (1959)
306. Lona, F., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 235 (1959)
307. Muller-Tholl, W. R., and Hartmann, W., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 275–76 (1959)
308. Napp-Zinn, K., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 278 (1959)
309. Picard, C., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 300 (1959); *Compt. rend.* (In press)
310. Purvis, O. N., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 314–15 (1959)
311. Razumov, V., *Trudy Priklad. Botan. Genet. i Selektsii*, 1, 10–18 (1958)
312. Razumov, V., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, **II**, 321 (1959)
313. Wellensiek, S. J., *Koninkl. Ned. Akad. Wetenschap., Proc., Ser. C*, **61**, 561–71 (1958)

*English translation will be announced in Technical Translations, issued by the Office of Technical Services, U.S. Dept. of Commerce, and will be made available by the Photoduplication Service, Library of Congress and by the SLA Translation Center at the John Crear Library, Chicago, Illinois.

THE MECHANISM OF ACTION OF AUXIN[1,2]

By A. W. Galston and W. K. Purves

Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

Introduction

More than 30 years have elapsed since the discovery of the auxins as natural growth promoters in higher plants. In all this time, despite numerous able and extensive research efforts, the primary mechanism of action of auxin has not been elucidated. This failure is undoubtedly due to the tremendous difficulties which stand in the way of such investigations; included among these are the extraordinarily low concentrations of auxins that suffice to produce the growth effects being measured and the probable subtlety of mechanism involved. In many respects the situation parallels that of animal hormones; their chemical nature has been known for many years, but their mode of action still eludes the biochemist.

Despite the lack of definitive information, the feeling has recently developed that the action of auxins can be reasonably well understood in terms of effects on the chemical composition of the cell walls. As we shall see below, there is indeed justification for this belief, from both physical and biochemical experiments. Nevertheless, the data supporting this point of view are by no means conclusive, and there is a sufficient literature on the effects of auxin on nonwall components of the cell to cause us to reject out of hand any "mechanism of action" which does not simultaneously take these effects into account.

Since we will not be able to define the mechanism of action of auxin, we are forced to attempt a balanced review of the abundant literature bearing on this problem. This subject has already been reviewed many times (1, 13, 14, 19, 32, 33, 103, 104, 159, 194, 276) and it will obviously not serve any useful purpose here to re-review all papers in this field. What we hope to do is to give some indication of the major kinds of explanations advanced, to judge each on the basis of the data available, and to arrive at some notion of the possible modes of action of auxin. We therefore wish to claim neither a complete literature coverage nor a successful definition of the object of our search. In many respects this review will be too long for the amount of definite information it conveys. To this we can only say that when the mechanism of action of auxin is more definitely known a complete review written on this subject will be much shorter, possibly even a single paragraph.

[1] The survey of literature pertaining to this review was concluded in June 1959.

[2] The following abbreviations will be used: DPD (diffusion pressure deficit); IAA (indole-3-acetic acid); NAA (α-naphthaleneacetic acid); OC (osmotic concentration); SH (sulfhydryl); TCA (trichloroacetic acid); TP (turgor pressure); 2,4-D (2,4-dichlorophenoxyacetic acid).

Definition of Terms and Scope of the Problem

Until several years ago, there was relatively little difficulty in defining auxins. As a committee of the American Society of Plant Physiologists declared, "Auxin is a generic term for compounds characterized by their ability to induce elongation in shoot cells. They resemble indole-3-acetic acid (IAA) in physiological action. Auxins may and generally do affect other processes besides elongation, but elongation is considered critical. Auxins are generally acids with an unsaturated cyclic nucleus or their derivatives" (285). Today this definition is no longer adequate because of the demonstration (169, 303) that gibberellins also exist in higher plants, and probably function as natural growth regulators. Such compounds are also characterized by their ability to induce elongation in shoot cells, in some respects resemble IAA in physiological action, do affect other processes besides elongation, and are acids which in some instances have an unsaturated cyclic nucleus. It will therefore be necessary for us to begin by distinguishing between auxins and gibberellins. This has been done by various workers, most recently Brian (43), but for the sake of convenience and completeness, a summary of differences will be repeated here (Table I).

TABLE I

A Summary of Major Differences Between Auxins and Gibberellins

	Auxin	*Gibberellin*
1. Transport polar	yes	no
2. Promote root initiation	yes	no
3. Inhibit root elongation	yes	no
4. Delay leaf abscission	yes	no
5. Inhibit lateral buds	yes	no
6. Induce callus formation	yes	no
7. Promote epinastic responses	yes	no
8. Promote growth of intact plants, especially of the dwarf type, and of monocotyledonous leaves	no	yes
9. Promote seed germination and the breaking of dormancy	no	yes
10. Promote bolting and flowering in nonvernalized biennials and in long-day plants	no	yes

In addition to the list of differentiating characteristics shown in Table I, auxins and gibberellins have many characteristics in common. Among these are promotion of elongation of cells of various test objects such as *Avena* coleoptiles and pea internodes and the induction of parthenocarpy in certain unfertilized ovaries.

Of the numerous effects known to be produced by auxin, only one class, that of the promotion of cell elongation, has been at all intensively studied with respect to mechanism of action. We must therefore limit ourselves to an analysis of this problem, although we admit at the outset that the effects

of auxin both in inducing mitotic activity and in differentiation of tissues may be significantly linked with the mechanism by which it induces cellular elongation.

Even with this limitation, there is a possible multiplicity of auxin action and it is difficult to separate the various effects from the elongation process itself. For convenience we shall list here the major effects known to be produced by auxins on elongating cells typically used in such experiments. Each of the topics will be discussed in greater detail below. The major effects are as follows: (*a*) increased cell wall plasticity; (*b*) increased water uptake; (*c*) altered permeability patterns; (*d*) decreased protoplasmic viscosity; (*e*) increased rate of protoplasmic streaming; (*f*) altered respiratory patterns; (*g*) altered nucleic acid metabolism. Before beginning a systematic discussion of each of these major effects, it will be instructive to consider the problem of the locale of auxin action.

Kinds of Cells Affected By Auxin

Although some promotive effects upon the growth of algal (271) and fungal (106) cells have been recorded in the literature, it is clear that the striking growth-promotive effects of auxin are largely limited to higher plant cells. These cells are best distinguished from all others by the possession of a unique complex of chemical components in the cell wall. These include cellulose, hemicelluloses, noncellulosic polysaccharides, pectic substances, lignin, and minor unknown components. Whether this is a significant fact, or whether it is merely a correlate of some other fundamental distinction between cells affected and cells not affected by auxin is not yet known. At the moment we can only mention it and suggest that there is something about this complex of substances (or one of them) in the cell wall that is essential for auxin action.

Not all higher plant cells, however, are sensitive to auxin. In some instances, this lack of sensitivity is due to the irreversible effects of heavy lignification or other structural immobilization of the protoplast or cell wall. In other instances, differences in auxin sensitivity appear to be due to more subtle biochemical limitations. Such failure to react to auxin may be due to: (*a*) prior exposure to unfavorable conditions of light or temperature or to anaerobiosis (133); (*b*) limitation by some other growth factor, such as adenine or kinetin (249), or unknown components of casein hydrolyzate or coconut milk (55, 259); (*c*) prevention of action of auxin, or inactivation due to the presence of inhibitors of growth (260) or to auxin-inactivating systems (89, 217, 309). The evidence seems strong that many inhibitors operate to keep cells in a state of rest or dormancy, preventing their response to auxin or to other growth promoters. For example, in timothy roots, various substituted coumarins have been shown to exist *in vivo* in concentrations that, when applied *in vitro*, cause a great inhibition of auxin-induced growth (100, 101). With regard to auxin-inactivating systems of plants, there is no

convincing evidence that they operate *in vivo* with the speed of *in vitro* preparations. However, several researchers have demonstrated decarboxylations (80, 81, 301), conjugations (7, 8, 99) and other *in vivo* inactivating transformations of applied auxins. In some instances (81) light has accelerated such degradative transformations.

As for the possible *in vivo* meaningfulness of the peroxidase type of IAA (indole-3-acetic acid) oxidase the following may be said: (*a*) Gradients of activity of this enzyme (89, 217) and of its inhibitor (86) show close inverse correlations with growth patterns in some tissues, but not in others. (*b*) In many instances, there is an inverse correlation between peroxidase activity and the growth habit of the plant. This is true for dwarf vs. tall plants (143, 237, 288), for bolting vs. nonbolting biennials (151), and for aerobically vs. anaerobically growing rice coleoptiles (309). (*c*) In certain organs, auxin content and IAA oxidase activity are inversely correlated (217). (*d*) The synthesis of the inhibitor of IAA oxidase is dependent on the red-far-red reversible photomorphogenic system known to exert a profound effect on growth (132) and to be possibly involved in auxin physiology (87, 165). Clearly, although none of this evidence compels the view that IAA oxidase operates significantly *in vivo,* the number and clarity of these correlations forces serious attention to be given to this possibility, as Ray (229) has already pointed out. Certainly, the situation canot be as lightly dismissed as it was in one previous review in this journal (37).

The Intracellular Localization of Auxin Action

We have already made it clear that the basic mechanism by which auxin induces cell elongation is as yet unknown. Can we at least delineate that portion of the cell with which auxin must interact to produce its responses? As early as 1930, a scant two years after the first publication of Went's (302) pioneer researches on auxin, Heyn (127) showed that in the *Avena* coleoptile, the growth promoting properties of auxin were associated with an increase of the plastic component of extensibility of the cell wall. Since such a mechanism could easily account for the known increase in growth induced by auxin, Heyn proposed this as a mechanism of auxin action. Many investigators in subsequent years (62, 63, 256, 267) have been able to corroborate this original report of Heyn, and the impression has grown that we must look to the cell wall for an explanation of the mechanism of auxin action.

It was clear from the original work of Heyn (127, 128) that auxin could not produce its effects on dead cell walls, so some intervention of the living portion of the cell is required. It was also clear from the work of Bonner (25) and of Heyn and Van Overbeek (130) that auxin can produce its effects on plasticization and on elongation at low temperatures (2 to 4°C.) at which no increase in the weight of cell wall material occurs. This leads to the conclusion that auxin acts on some protoplasmic system, this

action leading to an altered arrangement of cell wall components, this in turn leading to greater extensibility.

There is a danger, however, in fixing one's attention too firmly on the cell wall as the locale of auxin action. It has already been mentioned that auxin produces measurable effects on the cytoplasm of the cell, and in some instances on certain of the cell organelles. Among the effects noted are a decrease in cytoplasmic viscosity, an increase in the rate of protoplasmic streaming, increased oxygen consumption (in the light of present biochemical thinking, probably mitochondrial), altered permeability patterns, and effects on nucleic acid metabolism, presumably operating on the nucleus directly.

A direct experimental attack on this problem is of course difficult. Two general approaches seem possible. One is to apply auxin to cells from which one or more of the major potential sites of auxin action has been removed. The other is to apply labeled auxin, and then by fractionation of the cell to attempt to determine that portion of the cell into which the auxin has found its way. The first of these approaches has been followed by Thimann & Beth (271) in some recent experiments with the alga *Acetabularia*. This organism has been shown by Hämmerling (114) to contain a nucleus which migrates into the base of the stalk, at the apex of which is an umbrella-shaped appendage. The stalk may be cut so as to contain or to lack the nucleus. The rate of regeneration of the cut stalk, and of the formation of the umbrella, may then be studied in organisms possessing and lacking the nucleus. If the nucleus were the site of auxin action, as has been suggested (249), then extirpation of this organelle ought to prevent the promotive effect of auxin on growth. In the experiments of Thimann and Beth mentioned above, there was a persistent effect of auxin, even on the enucleated cells.

Although this would tend to lead one to examine locales other than the nucleus for an explanation of auxin action, it should be mentioned that *Acetabularia* is unique among organisms in the persistence of its nuclear products in the cytoplasm. Thus, although it is well known that *Amoeba* (189) cannot continue to synthesize proteins after its nucleus is removed, *Acetabularia* retains this ability for a long time, presumably due to the persistence of nuclear products (RNA?) in the cytoplasm. Thus the experimental results, however suggestive they may be, may actually not apply to more typical cells in which nuclear extirpation brings about a more dramatic decrease in growth and in protein synthesis.

The technique of applying labeled auxin to a cell and of studying the subsequent locale of the label, has not been extensively employed. Johnson & Bonner (142) applied C^{14}-carboxyl labeled 2,4-D (2,4-dichlorophenoxyacetic acid) of high specific activity to *Avena* coleoptile sections. They found the uptake to occur in three separable processes. The first, a diffusion type mechanism consummated in 20 to 30 min., involves the uptake of auxin

by a process that is readily reversible, in that the auxin may be readily leached to the outside solution. The second process, also rapid, resembles a binding to some cellular component, since this 2,4-D can be removed by exchange with unlabeled 2,4-D, but not by simple washing. The third component of the uptake is a continuing absorption, maintained at a steady rate for several hours, during which time the 2,4-D may actually be accumulated to concentrations higher than the external concentration. This appears to be similar to salt accumulation and the locale of such accumulation would presumably be the vacuole.

In somewhat similar experiments with pea stem sections, Galston & Kaur (92) have fed C^{14}-carboxyl labeled 2,4-D both to etiolated and to green sections and have then ground the tissue in 0.5 *M* sucrose +0.001 *M* ethylenediaminetetraacetic acid, and fractionated the homogenate into cell walls, chloroplasts (where present), mitochondria, microsomes, and a supernatant fraction. The etiolated sections, which grow optimally at 10^{-7} to 10^{-6} *M* 2,4-D, were being exposed to a great excess of the growth promoting compound. The presumptive site of auxin binding in the cell may therefore be completely obscured by an excess of auxin which is nonfunctional physiologically. With green sections, however, the growth increased with increasing auxin concentration up to and beyond 10^{-4} *M* 2,4-D. This material therefore constitutes a highly desirable object for this kind of study. In neither type of tissue could any evidence be found for the binding of auxin to any of the cell particulates. There was some evidence of the binding of auxin to a macromolecule in the final particle-free centrifugal supernatant, as shown by ammonium sulfate treatment, by boiling, and by dialysis experiments. Even in this case the bound radioactivity was so small as to make further experiments mandatory. The tentative conclusion from such studies is that auxin does not bind to any of the cell particulates, and that the locale of its action may thus be in the nonparticulate phase of the cytoplasm.

Direct evidence for this hypothesis was obtained in experiments in which attempts were made to precipitate, by boiling, the proteins of the final centrifugal supernatant fraction of control and of auxin-treated stem sections. This was done by pipetting aliquots of the final supernatant fractions into test tubes and immersing these, for 8 min., in a boiling water bath. The control tube yielded an abundant flocculent precipitate, while the 2,4-D-treated tube showed only a faint opalescence. Measurements of the quantity of heat-coagulated materials by turbidimetry or by weighing of the centrifuged precipitates yielded unequivocal evidence of decreased precipitates in the auxin-treated tubes. Although the physiological significance of this finding is not clear, the fact that both IAA and 2,4-D are effective in decreasing the heat coagulability of the final supernatant (proteins?) without decreasing their total yield via precipitation with 0.5 *M* trichloroacetic acid (TCA) shows that auxins can change the physical characteristics of cytoplasmic macromolecules (proteins?) just as they can change the physi-

cal nature of cell wall materials. This phenomenon will require much more investigation before its significance can be understood.

There is, of course, abundant evidence from kinetic experiments (32) and from structure-activity considerations (294) indicating an attachment of auxin to some receptor entity of the cell. For a variety of reasons, mostly circumstantial, it is felt that such a receptor entity is probably a protein. This conclusion has not yet been substantiated by the isolation of an unambiguous auxin-protein from growing plant cells, although such complexes have been demonstrated in the endosperm of cereal grain (102). Siegel & Galston (245) have reported the formation of an IAA-protein by pea roots which had been incubated for some hours in the presence of 10^{-3} *M* IAA (physiologically a very high concentration!). Their evidence, based on colorimetry, showed that when the roots which had been grown in the presence of the high concentrations of IAA were homogenized and the proteins deposited with trichloroacetic acid, these proteins possessed a pink color which is also given by IAA under similar conditions. When the proteins were treated with Salkowski reagent, they turned a bright pink, a reaction which is also characteristic of IAA. That this was not due to a simple adsorption of IAA onto protein, was shown by the mixing of control pea root protein *in vitro* with added IAA. Under these conditions no binding of auxin to protein occurred. It was also demonstrated that *in vivo* binding was dependent on aerobic metabolism and could be inhibited either by incubation with metabolic inhibitors or with another auxin which presumably competed with IAA for binding to the site mentioned.

The interpretation of the colorimetric data may, however, be more complicated than these simple observations would imply. Andreae & coworkers (7, 8, 99) have fed IAA to etiolated pea stems and roots, and after a suitable period of incubation have found all of the Salkowski positive material in the ethanol-soluble phase. From this phase they were able to isolate significant quantities of indoleacetylaspartic acid and indoleacetamide, as well as some free IAA, but could find no Salkowski-reacting proteins. They therefore suggest that the IAA-protein of Siegel and Galston is an artifact of the TCA-precipitation technique employed. Although this seems unlikely from the fact that control protein mixed with IAA and then precipitated with TCA did not yield a persistent pink color, the possibility must still be entertained that fed IAA gave rise to some of the conjugates mentioned above, and that these conjugates were then adsorbed into the proteins during precipitation, while IAA itself is not adsorbed under similar conditions. Alternatively, the ethanol extraction procedure used by Andreae *et al.* may have removed some form of bound IAA from a protein carrier. This question requires further clarification.

To summarize, there is no completely convincing evidence that indoleacetic acid or other auxins attach either to any particulate in the cell or to any macromolecule. There have been several suggestions of such attachment

that, however, may be open to other interpretations. The kinetic data indicating auxin attachment to some receptor entity in the cell still have no confirmation in actual experimental isolation of any auxin complex larger than the simple conjugates of Andreae & Good (7), although the auxin-induced alteration of the heat coagulability of the cytoplasmic proteins may be an indication of an auxin-protein union.

Effects on Cell Wall

Effects on cell wall plasticity.—As mentioned above, the early work of Heyn (reviewed by that author, 129) on the relationship between growth and the plastic extensibility of the cell wall is once again commanding a great deal of interest. Among the most important of Heyn's findings are these: (*a*) after decapitation of *Avena* coleoptiles, the plastic (irreversible) extensibility of the wall closely parallels the growth rate, while the elastic (reversible) extensibility shows no apparent relationship to the growth rate; and (*b*) the plasticity is greatly increased upon auxin treatment. The data on auxin and plasticity have been confirmed and extended by Cleland (62) and by Tagawa & Bonner (267). Cleland incubated *Avena* sections in IAA under conditions which prevented extension and showed that KCN given during the subsequent expansion period inhibited the growth and plasticity responses to IAA. The effects of IAA, potassium, calcium, and magnesium on plasticity and elasticity were studied by Tagawa & Bonner; the divalent ions decreased both wall properties, potassium increased the elasticity, IAA the plasticity, and a combination of potassium and IAA markedly increased both properties. Since certain physical properties of pectic substances are similarly affected by calcium and potassium, it was suggested that the plasticity and elasticity of the wall are dependent upon the pectic substances. Theories of auxin action based on pectic substances will be considered in a later section.

The existence of more than one phase of wall growth has been suggested by several investigators; perhaps the first of whom was Burström, whose views are summarized in his recent lecture (52). Burström's theory deals with root growth; but, as he has pointed out, similar phenomena are to be expected in shoot growth. He has postulated a minimum of two phases of growth, the first being a plastic stretching of the wall (promoted by auxin) and the second a calcium-dependent formation of new wall material (perhaps inhibited by auxin). The existence of "plasticization" and "rigidification" phases in cell walls seems to be well established (3, 54, 63), as does the plasticization of the wall by auxin. Busse (54) has shown that rigidification may be inhibited by cobalt, this effect becoming apparent only at the termination of auxin-induced growth. The action of auxin in controlling plasticization and rigidification appears relatively insensitive to anaerobiosis (2) and low temperatures (2, 130). The lack of effect of anaerobiosis is unexpected in view of the dependence of growth on aerobiosis (see below).

Chemistry of the cell wall.—Numerous schemes for the fractionation of

cell walls have been devised; some of these have been discussed by Bonner (31), and the scheme of Ordin *et al.* (213) has frequently been used. These methods have been useful, but all suffer from a lack of specificity for "pure" wall components. This difficulty has been avoided in the study by Bishop *et al.* (21), who subjected all of the fractions to hydrolysis followed by paper chromatography, thus definitely establishing the identities of the various components. Their study of the primary cell wall of the *Avena* coleoptile yielded these values for the various components: 51 per cent hemicelluloses (containing chiefly arabinose, galactose, glucose, and xylose residues); 25 per cent α-cellulose; 9 per cent proteins; and 4 per cent fat, waxes, and pigments. The ammonium oxalate-oxalic acid fraction, generally considered to be the "pectin fraction," made up 1.7 per cent of the dry weight; but this consisted primarily of a starch-like polysaccharide, so that only 0.3 per cent of the dry weight was pectic substances. This last figure is in sharp contrast with the higher values which have been obtained by other workers, e.g., 5 per cent (140) and 8 per cent (272). A critical comparison of the fractionation techniques employed in these investigations suggests that the value obtained by Jansen *et al.* (140), 5 per cent, may be the correct one, and that the low values obtained by Bishop *et al.* may be due to partial destruction of pectins.

Pectic substances.—Most investigations of the relationship between cell wall plasticity and the action of auxin have centered on the pectic substances, as was originally suggested by Van Overbeek (289). This emphasis has been occasioned by the apparent distribution of the pectic substances as a continuous phase in the cell wall (148) and by the physical and chemical properties of these substances (140, 149).

Buffel & Carlier (49, 56), and Wilson & Skoog (308) found that treatment with auxin of potato tuber and tobacco pith tissue led to an increased synthesis of pectic substances. These findings were corroborated by Albersheim & Bonner (5) in the *Avena* coleoptile; but Burström (53), studying wheat roots, found no effect of auxin on wall composition.

Other investigations have centered about the effects of auxin on the chemical state of the pectic substances or, more specifically, of the carboxyl group of the component galacturonic acid residues. In the insoluble pectic acid or pectate the carboxyl group is free or in the form of a salt of a divalent cation, but the carboxyl groups of soluble pectin are esterified by methyl groups. Ordin *et al.* (213, 214) reported that IAA enhanced the incorporation of methyl groups from methionine and of glucose carbon into a fraction identified as pectin ester. Jansen *et al.* (140), however, found that IAA, although it increased the turnover of methyl groups between methionine and pectic acid, caused no increase in pectin over pectic acid.

Effects of auxin on the apparent activity of pectin methyl esterase have been reported from several laboratories. Neely *et al.* (199) found that 2,4-D at growth-active concentrations caused a large increase in the activity of this enzyme; and Van Overbeek (292) suggested that this might be a

general effect of all auxins. In tobacco pith tissue, treatment with IAA induces an increase in pectin methyl esterase, a large fraction of which is bound to the cell wall (47). Since IAA had no effect on the *in vitro* activity of the enzyme, the effect of auxin was concluded to be on the synthesis or binding. The binding of pectin methyl esterase to cell walls of tobacco pith and artichoke tuber tissue has been investigated by Glasziou (94, 95, 96), who found that this binding was promoted by various auxins. Fractionation of the pectin methyl esterase from these two sources revealed the existence of at least three active enzymes, only one of which was influenced by auxin (97). It has been suggested by Glasziou that the growth-promoting activity of auxin results from an increased binding of pectin methyl esterase, thus protecting pectin from de-esterification and maintaining the wall in a plastic state. The findings of Yoda (317), although interpreted differently by that author, are consistent with those of Glasziou. On the other hand, work done by Jansen *et al.* (140a) suggests that this auxin-induced binding of pectin methyl esterase is a nonspecific effect and probably of little importance. In general, these reported effects of auxin on the attachment of enzymes to adsorbing surfaces are somewhat reminiscent of earlier reports on model systems involving charcoal and diastase (76 to 78), which were later shown not to be significant (255).

In this connection it is of interest that the reported effects of auxin on the methyl esterification of pectins in the wall can apparently not be confirmed in the laboratory of Bennet-Clark, one of the recent supporters of this theory (see Addendum).

Lignin.—Lignin is a rigid, inert, nonextensible component of plant cell walls (31). Thus, inhibition of lignification by auxin could conceivably serve as a mechanism of auxin-induced elongation. The chemistry of lignification has been reviewed by Siegel (244). In brief, it appears that derivatives of *p*-hydroxyphenylpropane are required as lignin precursors and that these residues are polymerized by peroxidases or other phenol oxidizing systems. In view of the dependence of lignin formation on peroxidations, one may envision an effect of auxin on lignification mediated by an effect on peroxidase level or activity. That auxin may inhibit the action of peroxidase *in vitro* has been shown by Siegel (243) and Ray (230), and more recent work by Siegel *et al.* (247) has shown that IAA can indeed inhibit peroxidase-mediated lignification in a model system. On the other hand, auxin has been shown to increase peroxidase activity when applied to plant tissue (141), and auxin treatment is thought to promote vascularization (137 to 139, 283, 304, 304a). The effect of auxin upon vascularization is, however, not too damaging to the possibility of auxin inhibition of lignification for two reasons. In the first place, vascularization is an exceedingly complex differentiation phenomenon of which lignification is but one aspect; secondly, IAA appears to oppose xylem differentiation in etiolated pea epicotyls (257). Solberg & Higinbotham (257) have suggested that the apparent promotive effect of auxin on vascularization in some systems may, in fact,

be a secondary one resulting from a hypothetical factor (other than auxin) produced during the auxin-induced promotion of meristematic activity.

Hemicelluloses, cellulose, and miscellaneous.—Unfortunately, virtually nothing is known of the physiology of the hemicelluloses, which are, according to Bishop *et al.* (21), the principal components of the *Avena* cell wall (51 per cent of the dry weight). α-Cellulose, which constitutes 25 per cent of the dry cell wall, is the second most important component on a weight basis.

The deposition of cellulose is proportional to the elongation of *Avena* coleoptile sections (272) and etiolated pea epicotyl sections (60) growing in the presence or absence of auxin; however, a study of growth responses to auxin at various temperatures indicates that there is no stoichiometry between auxin supply and cellulose deposition in *Avena* sections (272).

In an elegant kinetic study of the responses of *Avena* sections to auxin and cobalt, Busse (54) has suggested that auxin-induced growth may be limited by the deposition of cellulose and hemicelluloses in the cell wall. According to his hypothesis, auxin-induced growth is terminated by a sudden increase in the deposition of these rigid materials in the cell wall, and the growth promotion induced by cobalt results from an inhibition of this rigidification process. Also, Bayley & Setterfield (15) found that the walls of *Avena* sections grown in IAA contained a smaller percentage of cellulose than did the controls. Solberg & Higinbotham (257), studying the growth and differentiation of etiolated pea sections, noted that sucrose-induced growth was accompanied by an increase in wall thickness (apparently due to cellulose deposition) and that auxin opposed this action of sucrose. Boysen-Jensen (40) observed that epicotyl cells of *Phaseolus multiflorus* showed thin walls in the presence of excess auxin and thick ones when the auxin content was presumed to be low; from this he concluded that cellulose formation was closely related to auxin action and that auxin caused a "contraction" of the cellulose-synthesizing enzyme system by making bridges between the enzyme molecules. Although this theory is interesting it seems to go far beyond the data obtained.

The incorporation of C^{14} from labeled substrates into various *Avena* coleoptile cell wall fractions in the presence and absence of IAA was studied by Boroughs & Bonner (39). The only large effect of IAA was to increase by approximately 50 per cent the incorporation of label from acetate and sucrose into a fraction tentatively described as "noncellulosic polysaccharides." A 26 per cent reduction in incorporation from sucrose into "polyuronide hemicelluloses" may also have been significant. Nance (196) found that IAA altered the utilization of acetate-1-C^{14} by pea stem slices, favoring incorporation of label into organic acids and CO_2 at the expense of cell wall fractions. Perlis & Nance (216) reported that IAA enhanced the uptake of C^{14} from labeled pyruvate and acetate into "α-cellulose" and inhibited its incorporation into "pectin substances" and "polyuronide hemicelluloses."

Electron-dense bodies in the cell wall.—Newcomb & Siegesmund (203) have made a preliminary report on their study of the fine structure of the primary wall and cytoplasmic surface of tobacco pith cells cultured *in vitro*. Electron micrographs of thin sections of these cells characteristically showed numerous electron-dense bodies extending through the primary wall perpendicular to the cytoplasmic surface. These bodies, which were electron-dense even without osmium fixation, generally projected into the cytoplasm and appeared to consist of sacs bounded by membranes. Morphological changes in the wall occurred in the neighborhood of these sacs, and the authors interpreted these as indicating that the sacs may have modified wall growth. Treatment with auxin led to the appearance of strong striation in a "layer" of the wall adjacent to the cytoplasm, an increase in the number of certain electron-dense globular bodies found near the sacs, and the appearance of deeply staining masses of striae arising from the sac regions. These effects of auxin are of considerable interest but cannot be evaluated until further data are presented.

Effects on Water Uptake

A cell cannot grow without absorbing water. A vast number of changes occur during the growth of a cell, but an increased water uptake is the most characteristic and striking response to auxin; in fact, water uptake probably provides the motive force for cell enlargement. This increase must result either from a (perhaps transitory) change in one of the osmotic quantities, possibly mediated by effects on the cell wall, or from some form of "active" water uptake. These two possibilities will be considered below.

Osmotic quantities and effects of auxins.—According to the classical formulation (286), the net tendency of the cell to take up water from its environment [diffusion pressure deficit (DPD)] is a function of the osmotic concentration (OC) of the cell sap and of the turgor pressure (TP), (or wall pressure). An increased DPD can result from an increase in OC or from a decrease in TP.

Czaja (70) proposed that the effect of auxin was to increase the OC of the cell, thus raising the DPD and causing an increased water uptake. Many objections to Czaja's theory have been raised. In an earlier study, Ursprung & Blum (286) found that no increase in OC occurred in rapidly growing tissue; but their argument is not definitive, since an increased OC could be transient and overcome by the uptake of water. Hackett (107) showed that NAA (alpha-naphthaleneacetic acid) caused slight *reductions* in the OC of Jerusalem artichoke tuber tissue while promoting water uptake, and that this decrease in OC was explainable in terms of the increased water content of the tissue. Further evidence opposed to Czaja's theory has been obtained by other investigators (42, 162, 291).

Commoner *et al.* (66) suggested that the osmotic uptake of water might be activated by a respiration-linked salt uptake, which would increase

the OC of the cell sap. This theory is, of course, opposed by the findings cited above; but a stronger objection stems from Reinders' report (232) that auxin-induced growth can occur in distilled water. For a further discussion of auxin and OC, see Audus (13).

There is also the possibility that auxin may reduce the TP by some action on the wall; this would cause an increased DPD and, in turn, an increase in water uptake. As we have seen above, auxin can cause an increase in wall plasticity. If the wall is plasticized, it follows that the wall pressure and thus the TP of the cell are reduced, the DPD is increased, and water uptake is enhanced.

Transitory reductions in turgor pressure might be achieved by means other than a plasticization of the wall. Autonomous growth of the wall (i.e., the laying down of new structural material independent of water uptake) would result in a decreased wall pressure and TP (84). Thimann (270) has studied water uptake by potato discs after a period of osmotic inhibition; water uptake after the release of inhibition proceeded at a greater rate than in the controls, and this treatment did not alter the final amount of growth achieved. This effect could result either from autonomous growth of the wall or from plasticization during the period of osmotic inhibition (see, however, 269). On the other hand, Bayley & Setterfield (15) found that wall material was laid down in *Avena* sections only during active growth, and Bonner (25) reported that elongation need not be accompanied by wall synthesis. Incidentally, it need not follow from Frey-Wyssling's argument (84) that water uptake is the result, rather than the cause, of cell enlargement, as Kramer (152, p. 266) has suggested. This problem is, of course, largely a semantic one; but it seems obvious that, as long as a positive TP exists, a relaxation of the wall can hardly be considered the driving *force* in growth; rather this force is due to the inward diffusion of water.

Controversy over active water uptake.—The alternative to the theory that auxin promotes water uptake through an effect on osmotic quantities is that it is somehow involved in an "active" water uptake process. The question of the occurrence or nonoccurrence of active (nonosmotic) water uptake has been vigorously debated in recent years; fortunately, an excellent summary and analysis of the conflicting reports is to be found in the review by Kramer (152). Since this subject has been thoroughly reviewed (22, 22a, 23, 152, 153, 269, 270) only a brief summary need be presented here.

(*a*) Plasmolytic determinations of OC frequently yield higher values than do cryoscopic determinations [e.g., Bennet-Clark *et al.* (18a)], but Levitt (161) has suggested that the observed differences resulted from errors in measurement.

(*b*) Van Overbeek (290) observed that the exudate from tomato roots had a lower OC than did mannitol solutions which would inhibit exudation;

however, the interpretation of this result is open to question (9,211), since salt absorption from the sap and water dilution from non-xylary tissues may have occurred.

(*c*) Respiration must supply the energy required for any active water uptake process, and several workers have shown relationships between respiration and water intake. As will be shown in a later section, water uptake is strictly dependent upon aerobic metabolism and all inhibitors of respiration are inhibitory to water uptake. These results, however, need not be interpreted as evidence for nonosmotic water uptake; for, as Levitt (161) and Thimann (270) have suggested, the effects of auxin and respiratory inhibitors on water uptake are probably only secondary. Furthermore, Hackett & Thimann (113) have shown that water uptake is more sensitive to some inhibitors than is respiration, which would indicate that the link between the two processes is indirect.

(*d*) Bonner *et al.* (34) and Commoner *et al.* (66) have obtained evidence indicating that auxin-induced water uptake may occur even against an osmotic gradient. These experiments are, however, open to criticism on the ground that the supposedly nondiffusible external solutes employed are actually taken up by the test material (51b). Also, Burström (51b) has suggested that the OC value reported by Bonner *et al.* may have been too low and that tissue "adapts" to external OC by a modification of internal OC.

(*e*) Osmotic water losses are sometimes greater to a medium containing an electrolyte than to one with nonelectrolytes of the same OC (16, 41), but Levitt (163) claimed that this does not constitute evidence for an active water uptake mechanism since the water loss required several hours for completion.

(*f*) Leaf tissues plasmolyzed in sucrose solutions deplasmolyze under aerobic conditions (220); however, this need not indicate the occurrence of active water uptake, since Ordin *et al.* (211) have shown that a similar phenomenon in *Avena* sections can be explained in terms of penetration of the tissue by sucrose and subsequent osmotic uptake of water.

(*g*) Calculations by Levitt (161) indicated that a nonosmotic gradient of more than 1 or 2 atm. could not be maintained by the available respiratory energy. These calculations have been vigorously debated (16, 163, 164, 257a, 257b), and neither protagonist has been convinced by the arguments of the other.

Kramer (152) has concluded that "it seems possible that nonosmotic uptake of water occurs, but if so it is small in quantity and probably of little importance in plant water relations." If this conclusion is valid, as we believe it to be, then auxin-induced increases in water uptake must be explained primarily, if not entirely, in terms of altered osmotic quantities.

Differential effects of auxin on length and weight.—"Growth" is generally measured in one of two ways: as elongation, or as increase in fresh weight, both being primarily a reflection of water uptake. That these need

not be measures of the same fundamental processes has been indicated by a number of investigators. α-(*p*-Chlorophenoxy)-isobutyric acid causes a much greater increase in length than in fresh weight of wheat roots (51a), while benzimidazole induces more lateral than longitudinal growth in etiolated pea epicotyl sections (88). Iodoacetate is more effective in inhibiting growth in weight than growth in length of pea sections (60). Even more striking is the fact that in some systems the IAA optima for elongation and increase in fresh weight may differ by as much as two orders of magnitude (73, 90, 225). Under certain conditions these optima are differentially affected by the addition of sucrose (224). Further investigation of the auxin-sucrose interaction has led to the suggestion that auxin is involved in two distinct processes affecting the growth of pea sections: (*a*) it promotes water uptake but, (*b*) in the presence of high levels of sugars, it places a restraint upon the longitudinal extensibility of the cell wall. This hypothesis, along with the observed existence of a lag of several hours in the response to sugars, makes it possible to explain in simple terms the occurrence of different IAA optima for elongation and water uptake (224).

In summary, it appears that increased water uptake, which is of obvious importance in the growth response to auxin, must be explained in terms of an effect (or effects) of auxin on the osmotic relations of the cell. Specifically, it is likely that the turgor pressure is reduced either by wall plasticization or by deposition of new wall material. The interpretation of "growth" data in at least some tissues is complicated by differential effects of auxin on elongation and increase in fresh weight.

Membranes and Permeability

The fact that auxin produces obvious changes on the absorption of various materials by plant cells has led many investigators to propose direct auxin effects on the membrane as the primary mode of action of auxin. Originally proposed by Bungenberg de Jong & colleagues as a result of tests on *in vitro* phosphatide-type coacervate membranes (50, 51), the experiments were later extended by Veldstra & colleagues to oleate coacervates in which the turgescent action of auxins is roughly paralleled by their activity in the split pea curvature test (293, 295), and by Brian & Rideal (46) to phospholipids and lipoproteins. The evidence for the point of view that auxins affect membranes by virtue of their hydrophilic-lipophilic interfacial orientation has been previously reviewed in this series by Veldstra (294). It would now appear that the theory as originally proposed is inadequate because there are too many exceptions to the rules, in that many auxin-active molecules are either relatively or completely inactive in the coacervate test and vice versa.

Direct tests of the possible effect of auxin on the permeability of the cell membrane to water have been made by the use of deuterium- and tritium-labelled water (48, 150, 212, 277, 298). The general conclusion from

all of these papers is that auxin exerts relatively little effect on the permeability of the membrane to these compounds, too little to be considered as important in the prodigious effect of auxin on cell growth. Similar conclusions were reached by Hasman (116, 117) on the basis of studies on endosmosis and exosmosis in potato tuber cells. Some contrary evidence is found in the papers of Masuda (185 to 187) who claims that auxin increases the permeability of *Avena* coleoptiles cells to water and nonelectrolytes, while decreasing the "exchangeability" of intracellular Na with extracellular K. Meinl & von Guttenberg (189a) have also reported that auxin increases the permeability of the cell to water, and have suggested this as a means by which auxin can promote cell elongation. Bogen (22) however, has made the obvious point that an increased permeability can only reduce the time required for water equilibrium to be attained, but cannot alter the amount of water absorbed at equilibrium.

It has been established that auxin does promote the uptake of ions by various types of cells sensitive to its growth-promoting action (67, 115, 131, 187). In most, if not in all instances this effect must be considered secondary, since it can first be detected long after auxin action has been initiated.

Promotive effects of IAA on the uptake of amino acids has been demonstrated both in animal and plant cells (59, 233 to 235). Reinhold also found that sunflower hypocotyl sections incubated with physiological levels of auxin excreted tremendous quantities of ammonia. This effect could be counteracted by succinate or sucrose, indicating that auxin in some way led to an increased oxidation of amino acids, producing ammonia, and that this effect could be countered by supplying the cell with preferential substrates for oxidation.

Direct effects of auxin on the maintenance of differentially permeable membranes of the cells of excised bean endocarp have been observed by Sacher (238) and Sacher & Glasziou (239). In the absence of auxin, the cells lose their differential permeability and exude liquid into their normally air-filled intercellular spaces. This liquid contains large quantities of sucrose as well as pectins, amino acids, organic acids, and some enzymes. All of these changes can be prevented by the application of physiological levels of auxin 24 to 48 hrs. after excision of the bean tissue.

Thus, it appears clear that auxin can affect the permeability of cellular membranes to various substances. The data do not, however, in any way lead to the conclusion that this is the primary or even an important aspect of auxin action.

Protoplasmic Viscosity

Any theory of auxin action based on the cell wall must contend with the fact that very pronounced effects of auxin have been noted on the physical state of the cytoplasm. These results, first clearly obtained by Northen (207), have not been given adequate emphasis in subsequent auxin work though Audus (13) mentioned them in a review about a decade

ago. Because of this neglect and because current theories emphasize cell wall effects, it might be instructive to discuss in some detail Northen's simple and remarkably successful experiments. In most experiments, stems and petioles of 8- to 15-day-old navy bean plants were smeared unilaterally with lanolin or auxin-lanolin pastes. The treated tissues were then centrifuged at 680 × g. for 4 min., the tissue being so placed that the centrifugal force was perpendicular to the long axis of the cells. Freehand sections were then made and stained with I_2-KI. In all, some 4000 plants were treated and *ca.* 150,000 cells observed.

The results show clearly that unilateral applications of auxin in concentrations leading to negative curvature (growth acceleration at site of application), induce within 30 min. (and probably sooner) a marked decrease in viscosity of the cytoplasm of the cells on the treated side. For example, 30 min. after treatment the percentages of cortical cells with displaced contents were 65 and 52 on the treated side and only 15 and 14 on the control side. When the auxin concentrations were so high as to induce positive curvatures, these differences were obliterated. IAA, NAA, and indolepropionic acid were equally effective, and similar results were obtained with symmetrical apical placement of the lanolin-auxin mixtures on decapitated stems. It would certainly appear possible that these effects, which Northen ascribes to a dissociation of cellular proteins, represent either the basic action of auxin or one of its primary manifestations. Northen's interpretations are also supported by the earlier observations of Strugger (261) on the relation of protoplasmic viscosity to growth and by Stålfelt's (258) results on *Elodea*. It must be mentioned, however, that decreased protoplasmic viscosity may also be induced by many relatively nonspecific agents such as 0.6 *M* sucrose, 0.3 *M* KCl, low temperatures (3°C), ethylenechlorohydrin, thiourea, fat solvent anaesthetics (206, 208), and x-rays (209). Thus, if this demonstration of the action of auxin on protoplasmic viscosity is meaningful in the promotion of growth, then we must suppose that auxin operates via some specific mechanism of lowering protoplasmic viscosity. Certainly this phenomenon should be reinvestigated, and attempts should be made to achieve *in vitro* the effects already noted *in vivo*. The results of Galston & Kaur (92), demonstrating an auxin-induced alteration of the heat coagulability of cellular proteins, may, of course, represent an *in vitro* demonstration of the same phenomenon noted by Northen.

Protoplasmic Streaming

In many respects the action of auxin in lowering cytoplasmic viscosity is paralleled by its effect in increasing the velocity of cytoplasmic streaming. Indeed, the similarity of the action of light and of auxin and other chemical agents on these systems indicates that they are manifestations of the same basic phenomenon (85), and Eymers & Bottelier (75) suggested that streaming rate is directly proportional to the motive force causing streaming and inversely proportional to the viscosity.

Thimann & Sweeney (265, 266, 278) showed that IAA at 0.002–0.5 mg./l. (optimum 0.1 mg./l.) promoted streaming in *Avena* coleoptile cells. The effects appeared immediately and disappeared within 30 min., presumably due to exhaustion of carbon reserves since the addition of 1 per cent fructose (or other sugars) prolonged the effect for *ca.* 2 hr. The use of supraoptimal auxin concentrations depressed the streaming rate, and other auxin-active molecules produced effects similar to those caused by IAA. Although this auxin effect was later questioned (210), Sweeney (263) showed the objections to be invalid, and the negative results to be due to water infiltration of the tissue, resulting in anaerobic conditions. Showacre & DuBuy (242) later showed that even with adequate aeration, auxin could promote growth under conditions in which it failed to promote a streaming. This would indicate that streaming *per se* is not essential for auxin action. Sweeney (264) showed that IAA also promotes the streaming rate in *Avena* root hairs, the effective concentrations here being far below those needed for coleoptile cells. This correlation speaks in favor of the physiological significance of this phenomenon.

Recently, Radu (228) has found that adding IAA to the water that cereal grains are permitted to imbibe will enhance the streaming rate in the coleoptilar cells. Smirnova (254) has also shown that sulfhydryl groups are involved in protoplasmic streaming, since addition of $CdCl_2$ inhibits streaming, and subsequent addition of cysteine restores the rate to normal values. This observation is of interest because of previous theories on the involvement of sulfhydryl groups in primary auxin action (68).

Altered Respiratory Pattern

Relationships between auxin action and aerobic respiration.—A relationship between auxin action and oxidative metabolism was established in an early study by Bonner (24), who showed that *Avena* coleoptile sections could not grow under anaerobic conditions. The growth and auxin responses of *Avena* and pea sections, and of potato tuber discs, are markedly altered by differences in oxygen tension (108, 109, 111, 242, 273); however, the data of Hackett & Thimann (111) are hardly sufficient to constitute a "direct demonstration of the linear dependence of auxin functions upon aerobiosis," as Thimann & Leopold (276) have suggested.

The effects of respiratory inhibitors on auxin-induced growth also suggest a dependence of auxin action upon aerobic respiration. Bonner (24, 26) showed that cyanide inhibited the growth of *Avena* coleoptile sections; and the inhibitory action of carbon monoxide on *Avena* and pea sections has been conclusively demonstrated by Hackett & co-workers (108, 109). Other respiratory inhibitors which inhibit auxin-induced growth are arsenite, canavanine, fluoride, fluoroacetate, iodoacetate, and various organic arsenicals and mercurials (28 to 30, 36, 38, 60, 68, 274). Since the respiratory uncoupler, dinitrophenol, inhibits growth by almost 90 per cent while actually increasing oxygen uptake (28), it appears that the energy-rich com-

pounds produced by aerobic respiration are required for growth.

The relationship between auxin action and respiration appears to be reciprocal; although auxin action is possible only under aerobic conditions, treatment with auxin frequently results in an increased rate of oxygen consumption, as first shown by Bonner (26). Even though growth promotion induced by auxin is not always accompanied by an increased respiration rate, respiratory promotions in certain systems are well established (20, 26, 29, 61, 68, 112, 113, 190). The magnitude of the increase in respiration rate is generally, but not always, small and depends upon the conditions of the experiment. Typical values [tabulated by Thimann & Leopold (276)] are as follows: *Avena* coleoptile sections, 14 per cent (311); etiolated pea epicotyl sections, 20 per cent (61); potato tuber slices, 30 to 50 per cent (113); Jerusalem artichoke tuber tissue, 400 per cent (112).

Indirect nature of respiratory promotion by auxin.—Auxin does increase the respiration rate in many systems, but it seems probable that this promotion is an indirect one. The evidence for this conclusion has been discussed in detail by Bonner & Bandurski (33), and by Laties (157).

In general, the respiratory rate of fresh plant tissues is limited by the availability of phosphate acceptors because such tissues show little or no response to the addition of respiratory substrates, but they do show a pronounced stimulation of oxygen uptake by respiratory uncouplers such as dinitrophenol [see Table 1 in Laties, 1957 (157)]. The observation that respiration may be stimulated by exogenously supplied adenylate (29) also suggests limitation by the availability of phosphate acceptors. Increased activity of energy-consuming metabolic processes, which regenerate phosphate acceptors, results in an increase in respiration (15, 29, 61, 113, 115, 168, 236).

Auxin is without effect on respiration in the presence of dinitrophenol, arginine, or adenylate (29, 83; see, however 179). Similarly, dinitrophenol abolishes auxin-induced water uptake in a number of systems (29, 111, 113, 309); and arsenate, which uncouples phosphorylation during glycolysis as well as oxidative phosphorylations, inhibits auxin-induced growth and respiration in *Avena* coleoptile sections (30). From such data it has been deduced that auxin promotes water uptake through some energy-requiring process, thus increasing the level of phosphate acceptors and secondarily promoting respiration (see, however 179). This conclusion is supported by the fact that auxin promotes respiration only when growth is also promoted (29, 34, 68). Newcomb (202), however, has found that a respiratory promotion may be induced without a concomitant growth increase in tobacco pith tissue; but this may be reconciled with the preceding generalization if the energy-requiring process precedes the final step leading to growth.

The respiratory pathway related to growth.—It seems likely that in *Avena* coleoptiles and pea epicotyls at least two respiratory pathways are operative and that auxin-induced growth is dependent upon only one fraction of the total respiration; more specifically, this pathway seems to be

cytochrome oxidase-mediated and to involve the tricarboxylic acid cycle. Thimann and his co-workers have espoused this view ever since Commoner & Thimann (68) first suggested that the growth of *Avena* sections was intimately related to the metabolism of the four-carbon dicarboxylic acids. A discussion of some of the consequences of this theory is to be found in a lecture by Thimann (268). Malonate appeared to be as effective as malate in "reversing" the iodoacetate-induced inhibition of growth (273), and it was suggested that auxin could be regarded as a malonate derivative (268). However, Cooil (69) showed that many of the effects ascribed to the organic acids, including malonate, were in fact due to potassium ions. Under conditions where potassium was not limiting, relatively high concentrations of tricarboxylic cycle acids, but not malonate, could oppose the inhibitory action by iodoacetate; malonate, on the other hand, was inhibitory to growth.

Experiments on the sensitivity of growth to cyanide and carbon monoxide, and the light-reversibility of the carbon monoxide inhibition (24, 26, 108, 109) have led to the conclusion that the growth process is cytochrome oxidase-mediated. Studies on the effects of lowered oxygen tensions on water uptake are somewhat less conclusive (157).

That at least two respiratory pathways are active in some plants, and that only one of these is directly related to growth, is suggested by the selective effects of some respiratory inhibitors on growth, auxin-induced respiration, and basal respiration. For instance, iodoacetate abolishes the growth of *Avena* coleoptile sections at concentrations that inhibit respiration by less than 10 per cent (78). Comparable effects are also observed with arsenate (30), arsenite (61), canavanine (28, 29), and fluoride (36, 61). Further evidence for the existence of multiple respiratory pathways is to be found in studies of changing patterns of inhibitor-sensitivity with aging or "starvation" of tissues (4, 27, 111 to 113, 115, 156, 157). For a more detailed discussion of the evidence for the existence of multiple respiration pathways and the relationships between these pathways and growth, see the outstanding review by Laties (157).

Another possible effect of auxin on respiratory pathways was reported by Humphreys & Dugger (135), who observed that 2,4-D, but not IAA, increased the contribution to total respiration of the hexose monophosphate shunt as compared with the Embden-Meyerhof glycolytic pathway in pea roots with the cotyledons attached. It is not clear, however, that the activity did not result from a dichlorophenol impurity in the 2,4-D (98). On the other hand, Shaw *et al.* (241) found that IAA promoted monophosphate shunt respiration in wheat seedlings.

The effects of auxin on carbon dioxide uptake and of carbon dioxide on auxin-induced growth of *Avena* and pea sections have been studied by Yamaki (312, 313, 314). The addition of IAA to the growth medium results in a large, immediate reduction in the respiratory quotient as a consequence of an increase in oxygen consumption and a decrease in carbon dioxide

evolution, this in turn reflects a massive uptake of CO_2. That this auxin-induced CO_2 uptake may be intimately linked with the growth process is suggested by the fact that CO_2 stimulates growth, especially in the presence of exogenously supplied IAA. When the CO_2 tension is experimentally lowered, the growth responses are decreased. Mer *et al.* (189b, 189c) have also reported effects of CO_2 on the growth of *Avena* coleoptiles and first internodes, but have not studied the relation of this effect to auxin-induced growth.

Effects on oxidation-reduction systems.—Effects of auxin on various redox systems have been studied; of these investigations, perhaps the most thorough is that of the Milan group on the relationships between ascorbate, glutathione, and growth. Their initial observation that ascorbic acid inhibits growth and opposes the action of auxin (182, 280, 281, 282, 284, cf 205) has been attributed to the *in vivo* oxidation of ascorbate to dehydroascorbic acid (158, 180a, 182, 184, 284). It was further shown that auxin treatment favored the reduced over the oxidized form of ascorbate (11, 171, 173, 174, 284). These data led to the hypothesis that the physiological action of auxin results from its activity in reducing the level of the growth-inhibitory dehydroascorbic acid (173, 182, 279). Proceeding from this hypothesis, the Milan group suggested that the relationship between ascorbate oxidation level and growth might be explainable in terms of the oxidation level of glutathione, which is coupled to ascorbate by dehydroascorbic reductase (170, 181, 310) and appears to be widely involved in the control of enzyme activity. Marrè & Laudi (183) found that the decline in growth rate of decapitated pea seedlings was paralleled by a shift to the oxidized forms of ascorbate and glutathione; on the other hand, auxin at growth-promoting levels caused a shift toward reduced glutathione in *Avena* and pea sections (175). It has also been shown that oxidized glutathione inhibits growth (175) and that reduced glutathione can promote growth under certain conditions (175, 176, 205). The theory which has emerged from these data is that auxin promotes growth by favoring the reduced form of glutathione, an action resulting from auxin-induced inhibition of ascorbic acid oxidase (11, 173, 174, 193, 296). Inhibition of growth by supraoptimal levels of auxin is attributed to an inhibition of "monodehydroascorbate reductase" (177).

While this theory and its supporting data are of considerable interest, several objections may be raised. The first is that what has been demonstrated is a correlation, not necessarily a causal relationship. It is obvious that all of these effects on ascorbate and glutathione might as well be results of the growth process rather than causes of it, and kinetic studies (175, 180) are at least as compatible with the former view as with the latter. Marrè *et al.* (178) observed that the auxin dose-response curve for growth promotion is paralleled by changes in the ascorbate oxidation level but not by that in the glutathione oxidation level, which continues a trend toward the reduced state even at growth-inhibitory levels of IAA. This observation is

difficult to interpret, especially in view of the report that reduced glutathione promotes growth only in the presence of supraoptimal IAA levels (176). Further, it should be noted that ascorbic acid oxidase and dehydroascorbate reductase are far from the only enzymes inhibitable by auxins [e.g., Table II in Bonner & Bandurski (33)] and that ascorbic acid oxidase activity is increased by auxin in tobacco pith tissue (201). Also, Chinoy *et al.* (58) have reported that ascorbic acid promotes the growth of *Avena* coleoptile sections.

The findings of a group of Czechoslovak workers are of great interest, especially in view of the correlations between auxin action and ascorbic acid established by Chinoy *et al.* and the Milan group. An interesting compound called ascorbigen has been discovered and partially characterized (155, 221 to 223); it is hydrolyzed to yield ascorbic acid under acid conditions and IAA under alkaline conditions. It is found in great abundance in growing regions and to a lesser extent in older tissues; its level is low in dormant buds, and rises with the break of dormancy. The interpretation of these provocative findings must await further data.

A relationship between auxin action and sulfhydryl compounds has been suggested by a variety of observations, including the work of the Milan group, above). Several sulfhydryl inhibitors (e.g., arsenite, iodoacetate, and coumarin) have been shown to inhibit the action of auxin on respiration, water uptake, and growth by elongation (68, 110 to 113, 274, 275); and an apparent complex between auxin and pea root protein is broken by CoA (245). Muir *et al.* (195) suggested that the ring in the auxin molecule was bound to a sulfhydryl group as one of the two points of attachment; Leopold (159, 160), on the other hand, has proposed that auxin forms a thiol ester with CoA. Slocum & Little (253), after studying the growth-promoting and growth-inhibiting properties of iodoacetate and arsenite, concluded that these compounds produced their effects by partially inhibiting the lipoic acid-mediated oxidative decarboxylation of pyruvate and α-ketoglutarate. They further suggested that a more or less favorable allocation of substrate carbon between structural compounds and energy production could be established by control of such oxidative decarboxylations and that maximum growth promotion by auxin would occur when this balance was at an optimum.

A different relationship between auxin and sulfhydryls has been proposed by Thimann (268), who pointed out that various data from his laboratory indicate a major role for a sulfhydryl-containing enzyme in growth. According to his theory, auxin promotes growth by protecting such an enzyme from inactivation. While this theory is consistent with many of the known facts, some valid objections have been raised [e.g., Cooil (69)]; and no compelling new evidence favors this view over the many alternate hypothesis available.

Certain enzyme systems capable of oxidizing IAA *in vitro* are themselves suscepible to regulation by auxin. Pretreatment of pea tissue with

IAA results in an increase in IAA oxidase activity, and Galston & Dalberg (89) have suggested that IAA oxidase is an adaptive enzyme induced by the presence of IAA. Also, IAA induces the formation of an IAA-destroying enzyme by *Polyporus versicolor* (79). On the other hand, 2,4-D treatment reduces the *in vivo* auxin destruction in certain species (125, 126, 166, 300). Treatment of *Vicia faba* roots with IAA results in an increased peroxidase activity (141), but IAA inhibits the *in vitro* activity of peroxidase preparations from *Omphalia* and horseradish (230, 243). Of interest in this context are reports of increased "peroxigenesis" as a result of IAA treatment (6, 219, 246).

Siegel *et al.* (247), on the basis of a series of studies with model systems, have proposed that IAA may function as an antioxidant in plant tissues and may inhibit peroxidase-mediated free-radical oxidative polymerizations. The system investigated involved the lignification process, which as suggested above, might be involved in the control of growth by auxin. On the other hand, antioxidant action of IAA could conceivably be involved in other mechanisms of auxin action. This interesting theory obviously requires considerably more documentation.

Another possible auxin effect on an oxidation-reduction system, which could be involved in the control of respiratory pathways, has been described by Yamazaki (315). It has been suggested that IAA passes through a series of oxidation stages in its reaction with H_2O_2 and peroxidase, each of these stages representing the loss of one electron (316). The finding that IAA can reduce cytochrome-*c* in the presence of H_2O_2 and peroxidase is explained in terms of cytochrome-*c* reduction by a free radical, formed by the oxidation of IAA by one equivalent of H_2O_2 (315). If such an IAA radical occurs *in vivo*, it is possible that it might be involved in auxin effects on respiratory pathways through the reduction of cytochrome-*c*.

Auxins and Nucleic Acid Metabolism

It has been proposed, mainly by Skoog & colleagues (249 to 251), that auxin exerts its effects on growth by virtue of a metabolic interaction with nucleic acids or their constituents. The evidence for this belief is as follows:

(*a*) The growth effects produced by auxin may, in several instances, be greatly modified by the application of adenine or related compounds. For example, in tobacco pith callus cultures, the auxin-adenine ratio seems to determine whether roots will be initiated (high ratio), whether buds will be initiated (low ratio) or whether growth will continue as a callus (intermediate ratio) (192, 252). Cells supplied with auxin alone will enlarge greatly; cells supplied with auxin and kinetin (6-furfurylaminopurine) will both enlarge and divide (71, 136, 198). This action of kinetin in promoting cell division is apparently auxin-dependent, since kinetin will not produce marked effects in the absence of exogenous auxin. In excised pea stems and *Avena* coleoptiles and other organs, adenine and kinetin also interact with auxin in various ways. For example, auxin-induced growth of excised

sections is modified by adenine, kinetin, or antipurines (88, 91, 191); the inhibition of lateral bud growth imposed by auxin is reversed by adenine, kinetin, and certain purines (91, 306); and the root-initiating activity of IAA (and subsequent growth of the roots) are enhanced by adenine (91) but opposed by kinetin (72).

(*b*) The application of auxin to tobacco pith callus cultures induces an increase in the DNA and RNA content of the cells. The similar application of 10^{-5} *M* IAA to corn roots inhibits growth and also inhibits the normal rise of DNA and RNA which occurs during growth (215, 248).

(*c*) The application of NAA to decapitated *Tradescantia* stems prevents promeristem nuclei in the inhibited lateral buds from developing from the diploid to the tetraploid content of DNA (197). Since this transition is apparently a prelude to the breaking of the inhibition imposed by basipetally migrating auxin, another connection between auxin and nucleic acids is established.

(*d*) The work of Masuda (188), implicating cellular RNA in auxin action by virtue of an auxin-induced increase in the cation-binding capacity of RNA, has already been mentioned above.

How significant are these auxin-nucleic acid correlations as causal mechanisms in growth? To what extent may they be secondary manifestations, along with numerous other chemical and enzymic changes noted by numerous authors, of some primary auxin action? Although Skoog (249, pp. 12–13) believes them to be of primary importance, there is as yet no convincing evidence that they are more basic than the other auxin effects noted above.

Auxin and Its Receptor

Attention has been directed, in several previous reviews in the series, to the possibility that auxin attaches to some receptor entity in the cell (33, 294). The evidence for this belief is roughly as follows:

(*a*) A tabulation of the structural requirements for auxin action reveals a multiplicity of necessary molecular features (294). These include an unsaturated ring, bearing a side chain of certain length terminating in a potential or actual carboxyl group. The only exception to this rule is S-(carboxymethyl)-dimethyldithiocarbamate, which does not possess a ring, but which may become cyclic *in vivo* (105, 287). Depending on the nature of the ring and the length of the side chain, the ring positions must be either free or occupied. For example, in the phenoxy-acid series, at least one free ortho position is required for activity, but in the benzoic acid series, both ortho positions must be filled for the molecule to be active (294). These multiple requirements have led various workers to propose that auxin must attach to some receptor with appropriate spatial accomodations for the required groups on the auxin molecule.

(*b*) The kinetics of auxin action on *Avena* coleoptiles have been interpreted in terms of Michaelis-Menten enzyme kinetics (82) leading to the

necessary belief in the attachment between auxin and another entity. However, the basis on which this work rests, i. e., the constancy of coleoptile section growth rate with time, has been challenged (18). Although the objections raised seem to have been satisfactorily met (35) there continues to be a theoretical impediment to the use of a scheme developed for *in vitro* enzyme-substrate phenomena in so complicated a process as the promotion of growth of an intact plant cell by an exogenously applied substance. Another objection to the great dependence placed on auxin dose-response curves has been the demonstration that, in etiolated pea epicotyl sections at least, the existence of an auxin optimum for growth is completely an artifact dependant upon exogenously applied sucrose (224). Thus, if no sucrose is supplied to pea sections, no auxin optimum appears in the range 10^{-8} to 10^{-4} *M* IAA. If, however, sucrose is applied, an optimum appears in the range 10^{-7} to 10^{-6} *M*, depending on the type of section employed. The exact position of the optimum, as well as the magnitude of the subsequent inhibition, depends on the sucrose concentration employed. Sucrose also appears to potentiate both the promotive and inhibitive actions of auxin. Whether an auxin promotes or inhibits the growth of pea sections also seems to depend on when after excision and for how long the tissue is exposed to the auxin (224). Certainly, in view of these complications, kinetic results should be applied only with great caution to an analysis of the mechanism of auxin action.

(*c*) When exogenous auxins have been fed to tissue, various conjugates have been detected after a short period of incubation (*7*, 8, 99). Conjugates that have been detected are either greatly diminished in their auxin activity or entirely inactive, so one cannot properly consider them as functional in promoting growth.

The auxin receptor has generally been assumed to be a protein for the following reasons: (*a*) The concentrations of auxin effective in growth are so low as to make its action probably that of a coenzyme or enzyme activator. (*b*) Some auxin-protein complexes have been reported, though their significance remains obscure (102, 172, 245, 307). (*c*) The structural requirements of auxin-active molecules are consistent with the formation of peptidic bonds and linkage through sulfhydryl groups (194). In addition, some evidence has been adduced in favor of the participation of sulfhydryl groups, possibly protein-bound, in the growth response (68). This evidence, although suggestive, will remain no more than an attractive possibility until an unambiguous auxin-protein is isolated and its possible relation to growth phenomena carefully examined.

Auxins and Chelation

Several authors have found that various compounds with chelating activity are active as auxins. These compounds include ethylenediaminetetraacetic acid, diethyldithiocarbamate, 8-hydroxyquinoline, and S-carboxymethyl dimethyldithiocarbamate (17, 57, 122, 123, 200, 299). This

knowledge led Bennet-Clark to suggest (17) that auxins might act by virtue of their chelating properties. Cohen *et al.* (65) were able to demonstrate IAA-Cu and NAA-Cu complexes *in vitro* by spectrophotometric and titrational techniques. These findings were confirmed by Recaldin & Heath (231); however, they preferred to attribute the chelational effects to breakdown products of IAA, rather than to IAA itself. Armarego *et al.* (10) dispute all such reports, claiming that the "complex" is nothing more than the metallic salt of the auxin acid. Whatever the outcome of this dispute, it is clear that auxin action cannot be attributed exclusively to chelation, since there appears to be no direct correlation between chelation properties and auxin activity. It is still possible, however, that chelation represents one facet of auxin action, as suggested by Heath & Clark (124).

Interactions of Auxins with Gibberellins

The relationship between auxins and gibberellins has been discussed at considerable length by Brian (43) and by Purves & Hillman (226); therefore, only a brief summary of this problem will be presented here.

Synergistic interactions between auxins and gibberellins have been reported in several systems (12, 44, 45, 64, 74, 93, 118, 120, 154, 167, 227, 240, 297, 305); but in isolated sections of *Avena* coleoptiles and of etiolated pea epicotyls, the responses to gibberellin and auxin supplied simultaneously are, at most, simply additive (118, 120, 144, 145, 225, 226).

Kuse (154) has demonstrated that auxin must be present in order for petioles of *Ipomoea batatas* to respond to gibberellin, and similar results have been obtained by Brian & Hemming (44, 45) with green pea stem sections. It is possible that gibberellin may also be required for auxin-induced growth; but this may prove difficult to demonstrate, since we do not yet know whether gibberellin reserves may be experimentally depleted.

It has been suggested that gibberellin might act by means of an auxin-sparing mechanism, since gibberellin treatment has been observed to decrease the activities of certain presumed auxin-destroying systems in some plants and to increase the level of native auxin in another. Decreased IAA-oxidase activities as a result of gibberellin treatment have been found in some plant systems (86, 218, 220, 262) but not in others (45, 146, 147); and the activity of peroxidase, which can inactivate IAA *in vitro* (88a), has been found to be reduced by gibberellin treatment in dwarf, but not in normal, peas and corn (168a). This result was obtained with pyrogallol as a substrate; if similar experiments are conducted on rice with guaiacol as a substrate, then gibberellin appears to increase peroxidase activity (121). Nitsch (204) found that application of gibberellin to *Rhus typhina* caused an increase in the level of native auxin, but Hayashi & Murakami (119) found no effect on auxin levels in pea, tomato, and cucumber. Data prejudicial to the auxin-sparing theory of gibberellin action have been presented by Purves & Hillman (226).

Another suggestion (45, 93) is that a "third factor," perhaps an in-

hibitor, might be involved in the interaction of gibberellin and auxin. This theory has been favorably discussed by Brian (43), but the entire concept of auxin-mediated gibberellin action has been criticized (134, 226).

It seems fair to conclude that both auxin and gibberellin are required for cell growth, but that the nature of their interaction is not yet understood.

Addendum

Since the completion of this manuscript, both authors have been privileged to attend the Fourth International Conference on Plant Growth Regulation, held at the Boyce Thompson Institute for Plant Research, Yonkers, New York, from August 10 to 14, 1959. In view of the many contributions at the conference bearing on the subject matter of this review, we consider it useful to summarize some of them here briefly. All papers presented will be included in a volume to be published in 1960 by the Iowa State College Press, under the editorship of R. M. Klein.

Several new concepts of the relation between structure and activity of auxin molecules were presented. R. M. Muir and C. Hansch established correlations between auxin activity in the *Avena* section growth test and electron density distribution patterns (especially of π electrons) in the active molecule. The molecular orbital data were supplied and "superdelocalizability" calculations made by Professor Fukui in Japan. The correlations appeared to be as good as other correlations made previously, but no better. Muir and Hansch (supported by J. Bonner) continue to believe in covalent bond formation between auxin and some receptor.

In contrast with this point of view, most investigators have now shifted to a belief in some physical association, via appropriate charge-distribution patterns, between auxin and its receptor. For example, K. V. Thimann and W. Porter presented a theory of structure-activity relations based upon the presence in the active molecule of a strong negative charge, separated from a weaker positive charge by a distance of about 5.5 A. This simple description of activity seems to fit a great mass of data. V. Freed also presented data consistent with this type of theory. On the basis of several types of *in vitro* experiments, he considers than van der Waals and electrostatic forces may operate to achieve an auxin-protein association. Like Galston & Kaur (92), he found an effect of 2,4-D on the heat coagulability of a protein molecule, and was able to demonstrate that 2,4-D at 10^{-4} *M* depresses the fluorescence intensity of various enzymes without affecting their activity. Altered infrared absorption spectra in the region 825 to 780 cm^{-1} gave additional physical evidence for a physical interaction between auxin and protein. M. Bach has also independently found an effect of auxins on the heat coagulability of proteins.

J. Van Overbeek believes in a physical interaction between auxin and the lipoprotein cytoskeleton at the surface of cells. According to his theory, the ring portion of the active molecule is depressed in a "hole" in the lipophilic layer, leaving the carboxyl group projecting into an external hydrogen-bond

network. Specificity would depend on the manner of fit of the ring into appropriate holes in the cytoskeleton. R. L. Wain presented many detailed objections to this theory from the work of the Wye College group, however, and called for further data before a comprehensive theory is produced. With regard to auxin-pectin relations, T. Bennet-Clark reiterated that in his laboratory, no effects on pectin carboxyl and methoxyl could be detected during auxin-induced growth.

Several novel types of growth regulatory compounds were discussed, D. G. Crosby and A. J. Vlitos presented evidence that fatty alcohols present in Maryland Mammoth tobacco are the active "auxins" of this plant. Typical active compounds were 1-docosanol, octadecanol, and 2-heptadecanol. They report their inability to detect indolic auxins in this material. B. B. Stowe similarly reported on the growth promoting ability of fatty substances on excised pea stem sections. Active compounds include methyl esters, monoglycerides and triglycerides of C_{12} or heavier fatty acids, the fat-soluble vitamins E and K_1, various "Tweens" and phytol. He refrained from considering these compounds as auxins, because their promotive effects on growth were exerted only if auxin was also present. He believes them to operate via an enhancement of the activity of a cytochrome-*c* dependent respiratory system.

G. E. Blackman showed that *Lemna minor* accumulates labelled 2,4-D against a gradient and then re-excretes it into the medium. Other auxins behave similarly. There appear to be differences, in this re-excretion, between monocot and dicot behavior, a fact that may have some significance for herbicide mode of action. Bennet-Clark confirmed this phenomenon, adding that the re-excretion process can also occur against a gradient, although paradoxically it is not sensitive to anaerobiosis, cyanide, or azide. D. J. Osborne reported that 2,4-D delays senescence of leaf cells into which it penetrates, but hastens senescence in neighboring cells into which it does not pass.

L. J. Audus confirmed the previously reported "adaptation" of pea roots to applied auxins and apparent induction of a higher IAA oxidase activity by IAA and other compounds (89). However, he prefers not to attribute the adaptation to the induced changes in the enzyme. K. V. Thimann presented further evidence against the chelation theory of auxin action.

Several workers (J. A. Bentley, P. F. Wareing, and J. P. & C. Nitsch) described yet unknown naturally occurring growth promoters and inhibitors from a variety of sources, while A. M. Mayer and E. Marrè presented data supporting their contentions that (respectively) coumarin can act as an auxin, and ascorbic acid can counteract the effects of auxin. W. C. Hall presented evidence that C^{14}-labelled ethylene is absorbed, translocated as a water-soluble metabolite, and is then active in growth regulatory processes. Since ethylene occurs naturally in plant tissue, it would seem to qualify as a plant hormone. S. P. Burg and K. V. Thimann then presented evidence that ethylene production in apples is dependent on aerobic respiration. From

osmotic sensitivity data, they conclude that the reaction producing ethylene occurs in the mitochondria.

We shall not here review the numerous contributions on the gibberellins and on kinetin and its analogs, since most of the work presented does not bear directly on this review. We feel safe in concluding that, although provocative advances in auxin study are being made on many fronts, we are still a long way from answering any fundamental questions concerning its mode of action.

Conclusions

The mechanism of auxin action is not yet known.

The application of auxin to responsive plant cells results in a multitude of chemical and physical changes, none of which has yet been causally connected with the subsequent growth of the cell.

Current thinking emphasizes the importance of the role of auxin in increasing plasticization of the cell wall. While such effects are of obvious significance for cell elongation, there is no reason to consider them as more important than the known auxin-induced changes in the cytoplasm or nucleus.

Further advances in this field would seem to depend on satisfactory answers to one or more of the following questions, all of which can be attacked by currently available techniques:

(*a*) Where in the cell does auxin exert its primary action?

(*b*) Does it exert this action as the unchanged auxin molecule, or must it first be transformed to something else?

(*c*) Is there some common chemical basis for the known action of auxin on different parts of the cell, such as the wall, the cytoplasm, and the nucleus?

(*d*) Is the structural and kinetic evidence indicating the formation of a complex between auxin and some receptor conclusive? If so, could this complex be identical with already described entities such as indoleacetylaspartate or auxin-protein?

(*e*) Does auxin action depend upon the presence of other known growth regulators, such as gibberellin?

Literature Cited

1. Åberg, B., *Ann. Rev. Plant Physiol.*, **8**, 153–80 (1957)
2. Adamson, D., and Adamson, H., *Science*, **128**, 532–3 (1958)
3. Adamson, H., and Adamson, D., *Australian J. Biol. Sci.*, **10**, 435–42 (1957)
4. Albaum, H. G., and Eichel, B., *Am. J. Botany*, **30**, 18–22 (1943)
5. Albersheim, P., and Bonner, J., *J. Biol. Chem.* (In press)
6. Andreae, W. A., and Andreae, S. R., *Can. J. Botany*, **31**, 426–37 (1953)
7. Andreae, W. A., and Good, N. E., *Plant Physiol.*, **30**, 380–2 (1955)
8. Andreae, W. A., and Van Ysselstein, M. W. H., *Plant Physiol.*, **31**, 235–40 (1956)

9. Arisz, W. H., Helder, R. J., and Van Nie, R., *J. Exptl. Botany*, **2**, 257–97 (1951)
10. Armarego, W. L. F., Canny, M. J., and Cox, S. F., *Nature*, **183**, 1176–7 (1959)
11. Arrigoni, O., and Marrè, E., *Atti accad. nazl. Lincei. Rend., Classe sci. fis., mat. e nat.*, **17**, 390–4 (1954)
12. Asprey, G. J., Benson-Evans, K., and Lyon, A. G., *Nature*, **181**, 1351 (1958)
13. Audus, L. J., *Biol. Revs. Cambridge Phil. Soc.*, **24**, 51–93 (1949)
14. Audus, L. J., *Plant Growth Substances* (Leonard Hill, Ltd., London, England, 465 pp., 1953)
15. Bayley, S. T., and Setterfield, G., *Ann. Botany (London)*, **21**, 633–41 (1957)
16. Bennet-Clark, T. A., *Discussions Faraday Soc.*, No. 3, 134–9 (1948)
17. Bennet-Clark, T. A., in *The Chemistry and Mode of Action of Plant Growth* Substances, 284–91 (Wain, R. L., and Wightman, F., Eds., Butterworths Scientific Publications, London, England, 312 pp., 1956)
18. Bennet-Clark, T. A., in *The Chemistry and Mode of Action of Plant Growth Substances*, 310–2 (Wain, R. L., and Wightman, F., Eds., Butterworths Scientific Publications, London, England, 312 pp., 1956)
18a. Bennet-Clark, T. A., Greenwood, A. D., and Barker, J. W., *New Phytologist*, **35**, 277–91 (1936)
19. Bentley, J. A., *Ann. Rev. Plant Physol.*, **9**, 47–80 (1958)
20. Berger, J., Smith, P., and Avery, G. S., Jr., *Am. J. Botany*, **33**, 601–4 (1946)
21. Bishop, C. T., Bayley, S. T., and Setterfield, G., *Plant Physiol.*, **33**, 283–9 (1958)
22. Bogen, H. J., *Fortschr. Botan.*, **13**, 192–226 (1951)
22a. Bogen, H. J., *Planta*, **42**, 140–55 (1953)
23. Bogen, H. J., *Fortschr. Botan.*, **15**, 212–58 (1954)
24. Bonner, J., *J. Gen. Physiol.*, **17**, 63–76 (1933)
25. Bonner, J., *Proc. Natl. Acad. Sci. U.S.*, **20**, 393–7 (1934)
26. Bonner, J., *J. Gen. Physiol.*, **20**, 1–11 (1936)
27. Bonner, J., *Arch. Biochem.*, **17**, 311–26 (1948)
28. Bonner, J., *Am. J. Botany*, **36**, 323–32 (1949)
29. Bonner, J., *Am. J. Botany*, **36**, 429–36 (1949)
30. Bonner, J., *Plant Physiol.*, **25**, 181–4 (1950)
31. Bonner, J., *Plant Biochemistry* (Academic Press, Inc., New York, N.Y., 537 pp., 1950)
32. Bonner, J., *The Harvey Lectures*, **48**, 1–34 (1952–3)
33. Bonner, J., and Bandurski, R. S., *Ann. Rev. Plant Physiol.*, **3**, 59–86 (1952)
34. Bonner, J., Bandurski, R. S., and Millerd, A., *Physiol. Plantarum*, **6**, 511–22 (1953)
35. Bonner, J., and Foster, R. J., *J. Exptl. Botany*, **6**, 293–302 (1955)
36. Bonner, J., and Wildman, S. G., *Growth*, **10**, Suppl., 51 (1946)
37. Bonner, W. D., Jr., *Ann. Rev. Plant Physiol.*, **8**, 427–53 (1957)
38. Bonner, W. D., Jr., and Thimann, K. V., *Am. J. Botany* **37**, 66–75 (1950)
39. Boroughs, H., and Bonner, J., *Arch. Biochem. Biophys.*, **46**, 279–90 (1953)
40. Boysen-Jensen, P., *Biol. Medd. Kongelige Danske Videnskabernes Selskab (Copenhagen)*, **23**, No. 8, 51 pp. (1958)
41. Brauner, L., and Hasman, M., *Rev. fac. sci. univ. Istanbul, Ser. B*, **12**, 210 (1947)
42. Brauner, L., and Hasman, M., *Bull. fac. med. Istanbul*, **12**, 57–71 (1949)

43. Brian, P. W., *Biol. Revs. Cambridge Phil. Soc.*, **34**, 37–84 (1959)
44. Brian, P. W., and Hemming, H. G., *Nature*, **179**, 417 (1957)
45. Brian, P. W., and Hemming, H. G., *Ann. Botany (London)*, **22**, 1–17 (1958)
46. Brian, R. C., and Rideal, E. K., *Biochim. et Biophys. Acta*, **9**, 1–18 (1952)
47. Bryan, W. H., and Newcomb, E. H., *Physiol. Plantarum*, **7**, 290–7 (1954)
48. Buffel, K., *Vlaamse Akad. Wetenschap Belgie*, **14**, No. 7, 1–46 (1952)
49. Buffel, K., and Carlier, A., *Agricultura (Louvain)*, **4**, 157–76 (1956); *Chem. Abstr.*, **51**, 2952g (1957)
50. Bungenberg de Jong, H. G., and Bonner, J., *Protoplasma*, **24**, 198–218 (1935)
51. Bungenberg de Jong, H. G., and Saubert, G. G. P., *Protoplasma*, **28**, 352–359 (1937)
51a. Burström, H., *Physiol. Plantarum*, **4**, 199–208 (1951)
51b. Burström, H., *Physiol. Plantarum*, **6**, 685–91 (1953)
52. Burström, H., *Symposia Soc. Exptl. Biol.*, **11**, 44–62 (1957)
53. Burström, H., *Kgl. Fysiograf. Sällskap. Lund Förh.*, **28**, 53–64 (1958)
54. Busse, M., *Planta*, **53**, 25–44 (1959)
55. Caplin, S. M., *Am. J. Botany*, **43**, 749–54 (1956)
56. Carlier, A., and Buffel, K., *Acta Botan. Neerl.*, **4**, 551–564 (1955)
57. Carr, D. J., and Ng, E. K., *Physiol. Plantarum*, **12**, 264–74 (1959)
58. Chinoy, J. J., Grover, R., and Sirohi, G. S., *Physiol. Plantarum*, **10**, 92–9 (1957)
59. Christensen, H. N., Riggs, T. R., and Coyne, B. A., *J. Biol. Chem.*, **209**, 413–27 (1954)
60. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 230–47 (1950)
61. Christiansen, G. S., and Thimann, K. V., *Arch. Biochem.*, **26**, 248–59 (1950)
62. Cleland, R., *Physiol. Plantarum*, **11**, 599–609 (1958)
63. Cleland, R., and Bonner, J., *Plant Physiol.*, **31**, 350–4 (1956)
64. Clor, M. A., Currier, H. B., and Stocking, C. R., *Botan. Gaz.*, **120**, 80–7 (1958)
65. Cohen, D., Ginzburg, B.-Z., and Heitner-Wirguin, C., *Nature*, **181**, 686 (1958)
66. Commoner, B., Fogel, S., and Muller, W. H., *Am. J. Botany*, **30**, 23–8 (1943)
67. Commoner, B., and Mazia, D., *Plant Physiol.*, **17**, 682–5 (1942)
68. Commoner, B., and Thimann, K. V., *J. Gen. Physiol.*, **24**, 279–96 (1941)
69. Cooil, B. J., *Plant Physiol.*, **27**, 49–69 (1952)
70. Czaja, A. Th., *Ber. deut. botan. Ges.*, **53**, 221–45 (1935)
71. Das, N. K., Patau, K., and Skoog, F., *Physiol. Plantarum*, **9**, 640–51 (1956)
72. De Ropp, R. S., *Plant Physiol.*, **31**, 253–4 (1956)
73. De Ropp, R. S., Markley, E., *Plant Physiol.*, **30**, 210–4 (1955)
74. Dure, L. S., and Jensen, W. A., *Botan. Gaz.*, **118**, 254–61 (1957)
75. Eymers, J. G., and Bottelier, H. P., *Proc. Acad. Sci. Amsterdam*, **40**, 589–95 (1937)
76. Eyster, H. C., *Science*, **96**, 140–1 (1942)
77. Eyster, H. C., *Science*, **97**, 358 (1943)
78. Eyster, H. C., *Plant Physiol.*, **21**, 68–74 (1946)
79. Fåhraeus, G., and Tullander, V., *Physiol. Plantarum*, **9**, 494–501 (1956)
80. Fang, S. C., and Butts, J., *Plant Physiol.*, **32**, 253–9 (1957)
81. Fang, S. C., Theisen, P., and Butts, J. S., *Plant Physiol.*, **34**, 26–32 (1959)
82. Foster, R. J., McRae, D. H., and Bonner, J., *Proc. Natl. Acad. Sci. U.S.*, **38**, 1014–22 (1952)
83. French, R. C., and Beevers, H., *Am. J. Botany*, **40**, 660–6 (1953)

84. Frey-Wyssling, A., *Symposia Soc. Exptl. Biol.*, **6**, 320–8 (1952)
85. Galston, A. W., the *Encyclopedia of Plant Physiology*, **17**, 492–529 (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, 1959)
86. Galston, A. W., in *Photoperiodism and Related Phenomena in Plants and Animals* (Withrow, R. B., Ed., American Association for the Advancement of Science, Washington, D.C., 1959)
87. Galston, A. W., and Baker, R. S., *Am. J. Botany*, **40**, 512–6 (1953)
88. Galston, A. W., Baker, R. S., and King, J. W., *Physiol. Plantarum*, **6**, 863–872 (1953)
88a. Galston, A. W., Bonner, J., and Baker, R. S., *Arch. Biochem. Biophys.*, **49**, 456–70 (1953)
89. Galston, A. W., and Dalberg, L. Y., *Am. J. Botany*, **41**, 373–80 (1954)
90. Galston, A. W., and Hand, M. E., *Am. J. Botany*, **36**, 85–94 (1949)
91. Galston, A. W., and Hand, M. E., *Arch. Biochem.*, **22**, 434–43 (1949)
92. Galston, A. W., and Kaur, R., in *Proc. Intern. Botan. Congr., 9th Congr., Montreal, 1959*, **2**, 129 (Montréal, Canada, 1959); *Proc. Natl. Acad. Sci. U.S.*, **45**, 1587–90 (1959)
93. Galston, A. W., and Warburg, H., *Plant Physiol.*, **34**, 16–22 (1959)
94. Glasziou, K. T., *Australian J. Biol. Sci.*, **10**, 337–41 (1957)
95. Glasziou, K. T., *Australian J. Biol. Sci.*, **10**, 426–34 (1957)
96. Glasziou, K. T., *Nature*, **181**, 428–9 (1958)
97. Glasziou, K. T., and Inglis, S. D., *Australian J. Biol. Sci.*, **11**, 127–41 (1958)
98. Goldacre, P. L., Galston, A. W., and Weintraub, R. L., *Arch. Biochem. Biophys.*, **43**, 358–73 (1953)
99. Good, N. E., Andreae, W. A., and Van Ysselstein, M. W. H., *Plant Physiol.*, **31**, 231–5 (1956)
100. Goodwin, R. H., and Kavanagh, F., *Bull. Torrey Botan. Club*, **76**, 255–65 (1949)
101. Goodwin, R. H., and Taves, C., *Am. J. Botany*, **37**, 224–31 (1950)
102. Gordon, S. A., *Am. J. Botany*, **33**, 160–9 (1949)
103. Gordon, S. A., in *Growth and Differentiation in Plants*, 253–81 (Loomis, W. E., Ed., Iowa State College Press, Ames, Iowa, 458 pp., 1953)
104. Gordon, S. A., *Ann. Rev. Plant Physiol.*, **5**, 341–78 (1954)
105. Gordon, S. A., and Moss, R. A., *Physiol. Plantarum*, **11**, 208–14 (1958)
106. Gruen, H., *Ann. Rev. Plant Physiol.*, **10**, 405–40 (1959)
107. Hackett, D. P., *Plant Physiol.*, **27**, 279–84 (1952)
108. Hackett, D. P., and Schneiderman, H. A., *Arch. Biochem. Biophys.*, **47**, 190–204 (1953)
109. Hackett, D. P., Schneiderman, H. A. ,and Thimann, K. V., *Arch. Biochem. Biophys.*, **47**, 205–14 (1953)
110. Hackett, D. P., and Thimann, K. V., *Plant Physiol.*, **25**, 648–52 (1950)
111. Hackett, D. P., and Thimann, K. V., *Am. J. Botany*, **39**, 553–60 (1952)
112. Hackett, D. P., and Thimann, K. V., *Proc. Natl. Acad. Sci. U.S.*, **38**, 770–5 (1952)
113. Hackett, D. P., and Thimann, K. V., *Am. J. Botany*, **40**, 183–8 (1953)
114. Hämmerling, J., *Arch. mikroskop. Anat. Entwicklungsmech.*, **132**, 424–62 (1934)
115. Hanson, J. B., and Bonner, J., *Am. J. Botany*, **41**, 702–10 (1954)
116. Hasman, M., *Rev. fac. sci. univ. Istanbul, Ser. C*, **23**, 123–38 (1958)

117. Hasman, M., *Naturwissenschaften*, **23**, 573 (1958)
118. Hayashi, T., and Murakami, Y., *Nippon Nôgei-kagaku Kaishi*, **27**, 675–80 (1953)
119. Hayashi, T., and Murakami, Y., in *Proceedings of First Symposium on Gibberellin Research in Japan*, 1–2 (Japanese Gibberellin Research Association, Tokyo, Japan, 94 pp., 1957)
120. Hayashi, T., and Murakami, Y., *Nôgyô Gijutsu Kenkyûjo Hôkoku, D*, **7**, 159–97 (1958)
121. Hayashi, T., Murakami, Y., and Matsunaka, S., *Bull. Agr. Chem. Soc. Japan*, **20**, 159–64 (1956)
122. Heath, O. V. S., and Clark, J. E., *Nature*, **177**, 1118 (1956)
123. Heath, O. V. S., and Clark, J. E., *Nature*, **178**, 600 (1956)
124. Heath, O. V. S., and Clark, J. E., *Nature*, **183**, 1177 (1959)
125. Henderson, J. H. M., and Deese, D. C., *Nature*, **174**, 967 (1954)
126. Henderson, J. H. M., Miller, I. H., and Deese, D. C., *Science*, **120**, 710–2 (1954)
127. Heyn, A. N. J., *Proc. Acad. Sci. Amsterdam*, **33**, 1045–58 (1930)
128. Heyn, A. N. J., *Rec. trav. botan. neerl.*, **28**, 113–244 (1931)
129. Heyn, A. N. J., *Botan. Rev.*, **6**, 515–74 (1940)
130. Heyn, A. N. J., and Van Overbeek, J., *Proc. Acad. Sci. Amsterdam*, **34**, 1190–5 (1931)
131. Higinbotham, N., Latimer, H., and Eppley, R., *Science*, **118**, 243 (1953)
132. Hillman, W. S., and Galston, A. W., *Plant Physiol.*, **32**, 129–35 (1957)
133. Hillman, W. S., and Galston, A. W., in *Encyclopedia of Plant Physiology*, **14** (Ruhland, W., Ed., Springer-Verlag, Berlin, Germany, in press)
134. Hillman, W. S., and Purves, W. K., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
135. Humphreys, T. E., and Dugger, W. M., Jr., *Plant Physiol.*, **32**, 136–40 (1957)
136. Jablonski, J. R., and Skoog, F., *Physiol. Plantarum*, **7**, 16–24 (1954)
137. Jacobs, W. P., *Am. J. Botany*, **39**, 301–9 (1952)
138. Jacobs, W. P., *Am. Naturalist*, **90**, 163–9 (1956)
139. Jacobs, W. P., and Morrow, I. B., *Am. J. Botany*, **44**, 823–42 (1957)
140. Jansen, E. F., Jang, R., Albersheim, P., and Bonner, J., *Plant Physiol.* (In press)
140a. Jansen, E. F., Jang, R., and Bonner, J., *Plant Physiol.* (In press)
141. Jensen, W. A., *Plant Physiol.*, **30**, 426–32 (1955)
142. Johnson, M. P., and Bonner, J., *Physiol. Plantarum*, **9**, 102–18 (1956)
143. Kamerbeek, G. A., *Acta. Botan. neerl.*, **5**, 257–63 (1956)
144. Kato, J., *Mem. Coll. Sci. Univ. Kyoto, Ser. B*, **20**, 189–94 (1953)
145. Kato, J., *Physiol. Plantarum*, **11**, 10–5 (1958)
146. Kato, J., and Katsumi, M., *Naturwissenschaften*, **45**, 344 (1958)
147. Kato, J., and Katsumi, M., *Mem. Coll. Sci. Univ. Kyoto, Ser. B*, **26**, 53–60 (1959)
148. Kerr, T., in *Plant Growth Substances*, 37–42 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
149. Kertesz, Z. I., *The Pectic Substances* (Interscience Publishers, Inc., New York, N.Y., 628 pp., 1951)
150. Ketellapper, H. J., *Acta Botan. Neerl.*, **2**, 388–444 (1953)

151. Konishi, M., *Mem. Coll. Agr. Kyoto Univ., Botan. Ser. No. 3,* **75,** 1–70 (1956)
152. Kramer, P. J., *Ann. Rev. Plant Physiol.,* **6,** 253–72 (1955)
153. Kramer, P. J., and Currier, H. B., *Ann. Rev. Plant Physiol.,* **1,** 265–84 (1950)
154. Kuse, G., *Botan. Mag. (Tokyo),* **71,** 151–9 (1958)
155. Kutáček, M., Valenta, M., and Icha, R., *Experientia,* **13,** 284–6 (1957)
156. Laties, G. G., *Program Am. Soc. Plant Physiologists,* 36–7 (Gainesville, Fla., **1954)**
157. Laties, G. G., *Survey Biol. Progr., 3,* 215–99 (1957)
158. Laudi, G., *Nuova giorn. botan. ital.,* **62,** 368–73 (1955)
159. Leopold, A. C., *Auxins and Plant Growth* (University of California Press, Berkeley, Calif., 354 pp., 1955)
160. Leopold, A. C., and Guernsey, F. S., *Proc. Natl. Acad. Sci. U.S.,* **39,** 1105–11 (1953)
161. Levitt, J., *Plant Physiol.,* **22,** 514–25 (1947)
162. Levitt, J., *Plant Physiol.,* **23,** 505–15 (1948)
163. Levitt, J., *Physiol. Plantarum,* **6,** 240–52 (1953)
164. Levitt, J., *Physiol. Plantarum,* **7,** 592–4 (1954)
165. Liverman, J. L., and Bonner, J., *Proc. Natl. Acad. Sci. U.S.,* **39,** 905–16 (1953)
166. Lockhart, J. A., and Weintraub, R. L., *Am. J. Botany,* **44,** 424–8 (1957)
167. Luckwill, L. C., in *Cell, Organism, and Milieu; Seventeenth Growth Symposium* (Rudnick, D., Ed., Ronald Press Co., Inc., New York, N.Y., 1959)
168. Lundegårdh, H., *Arkiv Kemi,* **5,** 97–146 (1953)
168a. McCune, D. C., and Galston, A. W., *Plant Physiol.,* **34,** 416–18, 1959
169. MacMillan, J., and Sutter, P. J., *Naturwissenschaften,* **45,** 46 (1958)
170. Mapson, L. W., and Goodard, D. R., *Biochem. J.,* **49,** 592–601 (1951)
171. Marrè, E., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **16,** 758–63 (1954)
172. Marrè, E., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **18,** 88–94 (1955)
173. Marrè, E., and Arrigoni, O., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **18,** 539–47 (1955)
174. Marrè, E., and Arrigoni, O., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **19,** 320–4 (1955)
175. Marrè, E., and Arrigoni, O., *Physiol. Plantarum,* **10,** 289–301 (1957)
176. Marrè, E., and Arrigoni, O., *Atti acad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **22,** 641–9 (1957)
177. Marrè, E., and Arrigoni, O., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **23,** 454–9 (1957)
178. Marrè, E., Arrigoni, O., and Forti, G., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **22,** 85–91 (1957)
179. Marrè, E., and Forti, G., *Physiol. Plantarum,* **11,** 36–47 (1958)
180. Marrè, E., Forti, G., and Arrigoni, O., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **21,** 460–8 (1956)
180a. Marrè, E., Forti, G., and Pece, G., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **20,** 646–52 (1956)
181. Marrè, E., and Laudi, G., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **16,** 649–56 (1954)

182. Marrè, E., and Laudi, G., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.*, **20**, 77–82 (1956)
183. Marrè, E., and Laudi, G., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.*, **20**, 638–45 (1956)
184. Marrè, E., Laudi, G., and Arrigoni, O., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.*, **19**, 460–5 (1955)
185. Masuda, Y., *Physiol. Plantarum*, **8**, 527–37 (1955)
187. Masuda, Y., *J. Inst. Polytech. Osaka City Univ., Ser. D*, **9**, 1 (1958)
186. Masuda, Y., *Botan. Mag. (Tokyo)*, **70**, 62 (1957)
188. Masuda, Y., *Physiol. Plantarum*, **12**, 324–35 (1959)
189. Mazia, D., and Prescott, D. M., *Biochim. et Biophys. Acta*, **17**, 23–34 (1955)
189a. Meinl, G., and Guttenberg, H. von, *Planta*, **41**, 167–89 (1952)
189b. Mer, C. L., *Ann. Botany (London)*, **21**, 13–22 (1957)
189c. Mer, C. L., and Richards, F. J., *Nature*, **165**, 179 (1950)
190. Michel, B. E., *Botan. Gaz.*, **112**, 418–36 (1959)
191. Miller, C. O., *Proc. Soc. Exptl. Biol. Med.*, **83**, 561–5 (1953)
192. Miller, C. O., and Skoog, F., *Am. J. Botany*, **40**, 768–73 (1953)
193. Miller, I. H., and Burris, R. H., *Am. J. Botany*, **38**, 547–9 (1951)
194. Muir, R. M., and Hansch, C., *Ann. Rev. Plant Physiol.*, **6**, 157–76 (1955)
195. Muir, R. M., Hansch C., and Gallup, A. H., *Plant Physiol.*, **24**, 359–66 (1949)
196. Nance, J. F., *Plant Physiol.*, **33**, 93–8 (1958)
197. Naylor, J. M., *Cytological Studies of the Effects of Plant Growth Substances in Lateral Bud Inhibition* (Doctoral thesis, Univ. of Wisconsin, Madison, Wis., 1953)
198. Naylor, J. Sander, G., and Skoog, F., *Physiol Plantarum*, **7**, 25–9 (1954)
199. Neely, W. B., Ball, C. D., Hamner, C. L., and Sell, H. M., *Plant Physiol.*, **25**, 525–8 (1950)
200. Ng, E. K., and Carr, D. J., *Physiol. Plantarum*, **12**, 275–87 (1959)
201. Newcomb, E. H., *Proc. Soc. Exptl. Biol. Med.*, **76**, 504–9 (1951)
202. Newcomb, E. H., *Proc. Union intern. sci. biol., 1e coll. of intern. physiol. cultures de tissus végétaux*, 395–214 (Paris, France, 1954)
203. Newcomb, E. H., and Siegesmund, K. S., *Plant Physiol.*, **32**, Suppl., 19 (1957)
204. Nitsch, J. P., *Proc. Am. Soc. Hort. Sci.*, **70**, 512–25 (1957)
205. Nitsch, J. P., and Nitsch, C., *Plant Physiol.*, **31**, 94–111 (1956)
206. Northen, H. T., *Biodynamica*, **3**, 10–27 (1940)
207. Northen, H. T., *Botan. Gaz.*, **103**, 668–83 (1942)
208. Northen, H. T., *Plant Physiol.*, **21**, 148–54 (1946)
209. Northen, H. T., and MacVicar, R., *Biodynamica*, **3**, 28–32 (1940)
210. Olson, R. A., and DuBuy, H. G., *Am. J. Botany*, **27**, 392–401 (1940)
211. Ordin, L., Applewhite, T. H., and Bonner, J., *Plant Physiol.*, **31**, 44–53 (1956)
212. Ordin, L., and Bonner, J., *Plant Physiol.*, **31**, 53–7 (1956)
213. Ordin, L., Cleland, R., and Bonner, J., *Proc. Natl. Acad. Sci. U.S.*, **41**, 1023–9 (1955)
214. Ordin, L., Cleland, R., and Bonner, J., *Plant Physiol.*, **32**, 216–20 (1957)
215. Patau, K., Das, N. K., and Skoog, F., *Physiol. Plantarum*, **10**, 949–66 (1957)
216. Perlis, I. B., and Nance, J. F., *Plant Physiol.*, **31**, 451–5 (1956)
217. Pilet, P.-Ê., *Physiol. Plantarum*, **10**, 708–27 (1957)
218. Pilet, P.-Ê., *Compt. rend.*, **245**, 1327–8 (1957)
219. Pilet, P.-Ê., and Galston, A. W., *Physiol. Plantarum*, **8**, 888–98 (1955)

220. Pilet, P.-E., and Wurgler, W., *Bull. soc. botan. suisse*, **68**, 54–63 (1958)
221. Procházka, Z., Šanda, V., and Šorm, F., *Chem. listy*, **50**, 167 (1956)
222. Procházka, Z., Šanda, V., and Šorm, F., *Collection Czechoslov. Chem. Communs.*, **22**, 654–5 (1957)
223. Procházka, Z., Šanda, V., and Šorm, F., *Chem. listy*, **51**, 1197–8 (1957)
224. Purves, W. K., *Interaction of Sugar and Hormones in the Control of Growth of Etiolated Pea Epicotyl Sections* (Doctoral thesis, Yale Univ., New Haven, Conn., 1959)
225. Purves, W. K., and Hillman, W. S., *Physiol. Plantarum*, **11**, 29–35 (1958)
226. Purves, W. K., and Hillman, W. S., *Physiol. Plantarum* (In press)
227. Radley, M., *Ann. Botany (London)*, **22**, 297–307 (1958)
228. Radu, A., *Acad. rep. populare Romine (Sect. biol. arg.)*, **3**, 543–65 (1956)
229. Ray, P. M., *Ann. Rev. Plant Physiol.*, **9**, 81–118 (1958)
230. Ray, P. M., *Arch. Biochem. Biophys.* (In press)
231. Recaldin, D. A., and Heath, O. V. S., *Nature*, **182**, 539 (1958)
232. Reinders, D. E., *Rec. trav. botan. neerl.*, **39**, 1–140 (1942)
233. Reinhold, L., *Nature*, **182**, 1022–3 (1958)
234. Reinhold, L., and Powell, R. G., *Nature*, **177**, 658 (1956)
235. Reinhold, L., and Powell, R. G., *J. Exptl. Botany*, **9**, 82–96 (1958)
236. Robertson, R. N., and Turner, J. S., *Australian J. Exptl. Biol. Med. Sci.*, **23**, 63–73 (1945)
237. Ross, H., *Z. Induktive Abstammungs Z. Vererbungslehre*, **82**, 503–29 (1941)
238. Sacher, J. A., *Science*, **125**, 1199–200 (1957)
239. Sacher, J. A., and Glasziou, K. T., *Nature*, **183**, 757–8 (1959)
240. Schroeder, C. A., and Spector, C., *Science*, **126**, 701–2 (1957)
241. Shaw, M., Samborski, D. J., and Oaks, A., *Can. J. Botany*, **36**, 233–7 (1958)
242. Showacre, J. K., and DuBuy, H. G., *Am. J. Botany*, **34**, 175–82 (1947)
243. Siegel, S. M., *Physiol. Plantarum*, **7**, 41–50 (1954)
244. Siegel, S. M., *Quart. Rev. Biol.*, **31**, 1–18 (1956)
245. Siegel, S. M., and Galston, A. W., *Proc. Natl. Acad. Sci. U.S.*, **39**, 1111–8 (1953)
246. Siegel, S. M., and Galston, A. W., *Arch. Biochem. Biophys.*, **54**, 102–13 (1955)
247. Siegel, S. M., Frost, P., and Porto, F., *Plant Physiol.* (In press)
248. Silberger, J., and Skoog, F., *Science*, **118**, 443–4 (1953)
249. Skoog, F., *Brookhaven Symposia in Biol.*, **6**, 1–21 (1954)
250. Skoog, F., *Année biol.*, **31**, 201–13 (1955)
251. Skoog, F., and Miller, C. O., *Symposia Soc. Exptl. Biol.*, **11**, 118–31 (1957)
252. Skoog, F., and Tsui, C., *Am. J. Botany*, **35**, 782–7 (1948)
253. Slocum, D. H., and Little, J. E., *Plant Physiol.*, **32**, 192–6 (1957)
254. Smirnova, N. A., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **2**, 578–80 (1955)
255. Smith, F. G., Langeland, W. E., and Stotz, E., *Plant Physiol.*, **22**, 300–7 (1947)
256. Söding, H., *Jahrb. wiss. Botan.*, **79**, 231–55 (1934)
257. Solberg, R. A., and Higinbotham, N., *Am. J. Botany*, **44**, 769–78 (1957)
257a. Spanner, D. C., *Ann. Botany (London)*, **16**, 379–407 (1952)
257b. Spanner, D. C., *Physiol. Plantarum*, **7**, 278–82 (1954)
258. Stålfelt, M. G., *Exptl. Cell Research*, Suppl. **1**, 63 (1949)
259. Steward, F. C., and Caplin, S. M., *Science*, **113**, 518–20 (1951)
260. Steward, F. C., and Caplin, S. M., *Ann. Botany (London)*, **16**, 477–89 (1952)
261. Strugger, S., *Jahrb. wiss. Botan.*, **79**, 406–71 (1934)

262. Stutz, R. E., and Watanabe, R., *Semiannual Rept., Biol. Med. Research Div., Argonne Natl. Lab.*, **ANL-5732,** 107–9 (1957)
263. Sweeney, B. M., *Am. J. Botany,* **28,** 700–2 (1941)
264. Sweeney, B. M., *Am. J. Botany,* **31,** 78–80 (1944)
265. Sweeney, B. M., and Thimann, K. V., *J. Gen. Physiol.,* **21,** 439–61 (1938)
266. Sweeney, B. M., and Thimann, K. V., *J. Gen. Physiol.,* **25,** 841–54 (1942)
267. Tagawa, T., and Bonner, J., *Plant Physiol.,* **32,** 207–12 (1957)
268. Thimann, K. V., in *Plant Growth Substances,* 21–36 (Skoog, F., Ed., University of Wisconsin Press, Madison, Wis., 476 pp., 1951)
269. Thimann, K. V., *Growth,* **10,** Suppl., 5–22 (1951)
270. Thimann, K. V., *Am. Scientist,* **42,** 589–606 (1954)
271. Thimann, K. V., and Beth, K., *Nature,* **183,** 946–8 (1959)
272. Thimann, K. V., and Bonner, J., *Proc. Roy. Soc. (London) B,* **113,** 126–49 (1933)
273. Thimann, K. V., and Bonner, W. D., Jr., *Am. J. Botany,* **35,** 271–81 (1948)
274. Thimann, K. V., and Bonner, W. D., Jr., *Am. J. Botany,* **36,** 214–22 (1949)
275. Thimann, K. V., and Bonner, W. D., Jr., *Proc. Natl. Acad. Sci. U.S.,* **35,** 272–6 (1949)
276. Thimann, K. V., and Leopold, A. C., in *The Hormones,* III, 1–56 (Pincus, G., and Thimann, K. V., Eds., Academic Press, Inc., New York, N.Y., 1012 pp., (1955)
277. Thimann, K. V., and Samuel, E. W., *Proc. Natl. Acad. Sci. U.S.,* **41,** 1029–33 (1955)
278. Thimann, K. V., and Sweeney, B. M., *J. Gen. Physiol.,* **21,** 123–35 (1937)
279. Tonzig, S., and Marrè, E., *Rend. ist. lombardo sci., Pt. I,* **89,** 243–68 (1955)
280. Tonzig, S., and Trezzi, F., *Nuova giorn. botan. ital.,* **57,** 515–34 (1950)
281. Tonzig, S., and Trezzi, F., *Nouva giorn. botan. ital.* ,**57,** 535–48 (1950)
282. Tonzig, S., and Trezzi, F., *Atti accad. nazl. Lincci, Rend., Classe sci. fis., mat. e nat.,* **16,** 434–43 (1954)
283. Torrey, J. G., *Am. J. Botany,* **40,** 525–33 (1953)
284. Trezzi, F., *Atti accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.,* **21,** 210–25 (1956)
285. Tukey, H. B., Went, F. W., Muir, R. M., and Van Overbeek, J., *Plant Physiol.,* **29,** 307–8 (1954)
286. Ursprung, A., and Blum, G., *Jahrb. wiss. Botan.,* **63,** 1–110 (1924)
287. Van der Kerk, G. J. M., Van Raalte, M. H., Kaars Sijpestein, A., and Van der Veen, R., *Nature,* **176,** 308 (1955)
288. Van Overbeek, J., *Proc. Natl. Acad. Sci. U.S.,* **21,** 292–9 (1935)
289. Van Overbeek, J., *Botan. Rev.,* **5,** 655–81 (1939)
290. Van Overbeek, J., *Am. J. Botany,* **29,** 677–83 (1942)
291. Van Overbeek, J., *Am. J. Botany,* **31,** 265–9 (1944)
292. Van Overbeek, J., *Ann. Rev. Plant Physiol.,* **3,** 87–108 (1952)
293. Veldstra, H., *Biochim. et Biophys. Acta,* **1,** 364–78 (1947)
294. Veldstra, H., *Ann. Rev. Plant Physiol.,* **4,** 151–98 (1953)
295. Veldstra, H., and Booij, H. L., *Biochim. et Biophys. Acta,* **3,** 278–312 (1949)
296. Wagenknecht, A. C., Riker, A. J., Allen, T. C., and Burris, R. H., *Am. J. Botany,* **38,** 550-4 (1951)
297. Wareing, P. F., *Nature,* **181,** 1744 (1958)
298. Wartiovaara, V., *Acta Botan. Fennica,* **34,** 1–22 (1944)

299. Weinstein, L. H., Meiss, A. N., Uhler, R. L., and Purvis, E. R., *Nature*, **178**, 1188 (1956)
300. Weintraub, R. L., *J. Agr. Food Chem.*, **1**, 250–4 (1953)
301. Weintraub, R. L., Brown, J. W., Fields, M., and Rohan, J., *Plant Physiol.*, **27**, 293–301 (1952)
302. Went, F. W., *Rec. trav. botan. neerl.*, **25**, 1–116 (1928)
303. West, C. A., and Phinney, B. O., *J. Am. Chem. Soc.*, **81**, 2424–7 (1959)
304. Wetmore, R. H., *Science*, **121**, 626 (1955)
304a. Wetmore, R. H., and Sorokin, S., *J. Arnold Arboretum*, **36**, 305–17 (1955)
305. Whaley, W. G., and Kephart, J., *Plant Physiol.*, **32**, Suppl. 33–4, (1957)
306. Wickson, M., and Thimann, K. V., *Physiol. Plantarum*, **11**, 62–74 (1958)
307. Wildman, S. G., and Gordon, S. A., *Proc. Natl. Acad. Sci. U.S.*, **28**, 217–28 (1942)
308. Wilson, C. M., and Skoog, F., *Physiol. Plantarum*, **7**, 204–11 (1954)
309. Yamada, N., *Plant Physiol.*, **29**, 92–6 (1954)
310. Yamaguchi, M., and Joslyn, M. A., *Plant Physiol.*, **26**, 757–72 (1951)
311. Yamaki, T., *Misc. Repts. Research Inst. Nat. Resources (Tokyo)* No. 11, 37–40 (1948)
312. Yamaki, T., *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, **4**, 127–54 (1954)
313. Yamaki, T., *Plant Physiol.*, **31**, Suppl. 5 (1956)
314. Yamaki, T., *Plant Physiol.*, **32**, Suppl. 19 (1957)
315. Yamazaki, I., in *Proc. Intern. Symposium on Enzyme Chemistry*, 224–9 (Maruzen, Tokyo and Kyoto, Japan, 1958)
316. Yamazaki, I., *Arch. Biochem. Biophys.* (In press)
317. Yoda, S., *Botan. Mag. (Tokyo)*, **71**, 1–6 (1958)

SOME ASPECTS OF THE STRUCTURE AND BEHAVIOR OF TOBACCO MOSAIC VIRUS[1,2]

By Albert Siegel[3] and S. G. Wildman
Department of Botany, University of California, Los Angeles, California

Introduction

The remarkable elucidation of the structure of tobacco mosaic virus (TMV) in recent years, together with the demonstration that its nucleic acid moiety is sufficient to initiate infection, has sharply increased the tempo of research on this virus. An indication of the rapidity with which knowledge of TMV is accumulating is found in the fact that several excellent reviews (1 to 5) have appeared recently. In this discussion we will attempt to present an integrated view of the TMV infective process, starting with an account of the current knowledge of the structure and chemical composition of the virus and following this with an exposition of recent information on strain variation and induced mutation. The ensuing discussion will concern the intracellular events of TMV infection. We have started with the premise that the virus-caused disease is to be understood primarily in terms of the interactions between virus and single host cells and have, therefore, limited our coverage of the enormous TMV literature to those papers that have a direct bearing on this aspect of TMV virology.

Structure and Composition of TMV

Morphology of TMV.—TMV is a nucleoprotein of 40,000,000 mol. wt. (6, 7) with the overall shape of a cigarette. The linear dimension is 300 mμ (8 to 11). The outside of the cylinder appears to be grooved somewhat like a screw with a mean diameter of 150 A and maximum and minimum diameters of 180 A and 120 A, respectively (12). Five per cent of the virus is nucleic acid (13), and the remainder is protein. Metal atoms also appear to be intimately associated with the virus (14, 15). Many intimate structural details of the virus are known, and knowledge of the chemical composition of the virus is expanding rapidly.

The protein, in gross aspect, constitutes a molecular weight of 38,000,000 and is an ordered structure consisting of many identical subunits (16 to 18). There are somewhat more than 2000 such subunits, each having a molecular weight of about 18,000. The subunits are arranged in a helical array so as to present the aspect of a hollow cylinder, the pitch of the helix being 23°. Three turns of the helix contain 49 protein subunits and comprise the basic,

[1] The survey of literature pertaining to this review was concluded in August 1959.

[2] The following abbreviation will be used: TMV (tobacco mosaic virus).

[3] Present address: Department of Agricultural Biochemistry, College of Agriculture, University of Arizona, Tucson, Arizona.

69 A, repeat unit of the virus detected by x-ray crystallographic analysis (19).

The nucleic acid exists as a single polynucleotide strand (20 to 23) that follows the pitch of the protein helix. There are approximately three nucleotides per protein subunit. The determination of the radial density distribution of the virus (24, 25) has revealed the virus to have a hollow core 36 A thick that is surrounded by a protein cylinder with density concentrations at 24, 40, 66, and 78 A from the center. The nucleic acid is positioned at 40 A from the center, probably following a groove on the inside of the protein cylinder, so that the nucleic acid strand appears to be surrounded in all aspects by tightly packed, protein subunits.

Chemical composition of TMV protein.—The protein subunit of molecular weight 18,000—which had previously been inferred—has recently been

TABLE I

AMINO ACID COMPOSITION OF TMV PROTEIN SUBUNITS (29, 31)

Amino acid	Number of residues	Amino acid	Number of residues	Amino acid	Number of residues
Serine	18	Arginine	11	Aspartic acid	7
Threonine	17	Glutamine	9	Glycine	6
Alanine	15	Isoleucine	9	Tyrosine	4
Valine	14	Phenylalanine	8	Lysine	2
Leucine	13	Proline	8	Tryptophan	2
Asparagine	12	Glutamic acid	8	Cysteine	1

isolated in native form (26 to 28). Several investigators have explored the amino acid composition of the subunit with more accurate values being obtained as techniques have been refined. The most recent and independent estimates of Ramachandran (29) and Aach (30) are in fair agreement. The kind and number of amino acids contained in a subunit are found in Table I.

The amino acid sequence is also under intensive investigation. The work of Harris & Knight (32) with carboxypeptidase revealed that the C-terminal amino acid is threonine, while other investigators (33 to 35) have discovered that the last six amino acids in sequence are threonyl-seryl-glycyl-prolyl-alanyl-threonine. The N-terminal amino acid has been found to be acetylated, the sequence of the first two amino acids being acetyl-seryl-tyrosine (36, 37).

Gish *et al.* (38), Woody & Knight (39), and Schramm & Braunitzer (40) have subjected the protein subunits to tryptic digestion. Twelve peptides have been obtained in all and this number corresponds to the known specificity of trypsin, and to the number of arginine and lysine residues present in the subunit. Gish *et al.* (38), have determined that one of the

peptides, "I-peptide," which constitutes approximately 25 per cent of the protein subunit, is insoluble at pH 4.2 and contains only one of the eleven arginine residues present in the subunit. The other peptides have been isolated from each other by the method of counter-current distribution and the amino acid sequence has been determined for six of the eleven soluble peptides (41). Woody & Knight have separated the soluble peptides by the combined methods of paper electrophoresis and paper chromatography originally used by Ingraham (42) to "fingerprint" or "map" the peptides that can be obtained from a protein by different methods of degradation.

Structure and composition of TMV nucleic acid.—Evidence that the entire nucleic acid of TMV exists as a single polynucleotide chain lacking obvious repeat subunits is compelling and will be considered later in this review. Although the RNA chain is restricted in its configuration while contained within the virus, it can have either an ordered structure, in which about half of its nucleotides are involved in small helical regions (43), or it can exist as a random coil when free in solution. The protein-free nucleic acid may change its configuration reversibly, depending upon the environmental conditions; the presence of cations encourages the more ordered structure (44). Some evidence exists that there may be several discontinuities of structure in the RNA molecule because it appears to degrade spontaneously into preferred sizes (45).

The molar base ratio of TMV RNA has been found to be: adenine, 1.20; guanine, 1.01; cytosine, 0.74; and uracil, 1.06 (46). Slow progress is being made towards ascertaining the sequence of the bases along the RNA chain. An obstacle to success in this type of analysis is the very large number of nucleotides that must be kept in order as the molecule is degraded. Reddi & Knight (47) have subjected the nucleic acid to degradation by pancreatic ribonuclease, an enzyme that hydrolyzes the pyrimidine nucleoside phosphodiester bond at the C-5′ position. The enzyme does not hydrolyze the comparable purine nucleoside bond. Roughly half of the pyrimidine residues of the nucleic acid appear as monopyrimidine nucleotides in the enzymatic digest (48). From the known specificity of the enzyme, it can be inferred that each of the monopyrimidine nucleotides in the digest must have been preceded by a pyrimidine residue in the intact nucleic acid chain. It also follows that the half of the pyrimidine residues, which did not appear as mononucleotides in the digest, must have been preceded by a purine residue in the nucleic acid chain. Reddi (49) interprets these data, as well as data that concern the number of purine residues occurring in indigestible clusters of five or more, as signifying that the purines and pyrimidines are not distributed in an overall random manner in the nucleic acid chain but, rather, that they each tend to occur in clusters. Hart (50) has also analyzed the same data and, after considering a slow and poorly understood reaction which occurs during the digestion, has concluded that the purines and pyrimidines do have a random distribution in the nucleic

acid. Apparently, a firm decision in this matter will have to await further experimentation and analysis.

Reddi (51) has presented other evidence, however, indicating that whether or not the sequence appears to be random in gross aspect, the frequency with which particular microsequences occur departs markedly from chance. For instance, the sequence pyrimidine phosphate-guanosine phosphate-cytosine phosphate appears to be completely absent from the nucleic acid chain, although one would expect that in a random sequence 9 per cent of the guanylic acid residues and 12 per cent of the cytidylic acid residues of the chain would be involved in this trinucleotide sequence.

Although the study of nucleic acid is currently in a primitive state, it is to be expected that much future work will be devoted to unraveling the chemistry of this biologically important substance.

Infectivity of TMV nucleic acid.—The remarkable discovery (52, 53) that the nucleic acid moiety of TMV can initiate infection has been repeatedly confirmed (54, 55), as well as being extended to several of the RNA-containing animal viruses (56, 57) and plant viruses (58 to 60). Although it had been suspected for some time that the nucleic acid of the virus was the bearer of its genetic information, the demonstration that an infected cell could replicate viral nucleic acid as well as synthesize virus-specific protein when presented only with a nucleic acid molecule, provided definitive evidence that this was indeed the case.

There is general agreement among several workers (20, 21, 61) that only free nucleic acid molecules of the size found within the virus particles are infectious. Several kinds of experimental evidence suggest that rupture of any one of the 6000 phosphodiester bonds in the nucleic acid is sufficient to destroy infectivity (20, 22). Attempts to obtain infectivity with nucleic acid molecules smaller than those contained within the virus molecule have been unsuccessful except for one unconfirmed report (62).

Generally, nucleic acid preparations have a specific infectivity 0.1 per cent of that of intact virus preparations, or less, when they are compared on a nucleic acid weight basis. Nucleic acid fractions of higher activity have been obtained by passage of crude extracts through Ecteola columns (63). A difficulty exists in defining a specific activity for nucleic acid preparations because different values are obtained depending on the host species, age of plant, and age of leaf used in making the comparative assay (55, 64). The infectivity of a nucleic acid preparation can be increased to a marked extent by polymerizing viral protein fragments around the nucleic acid molecules (65, 66). The product thus obtained, reconstituted virus, has a marked resemblance to intact virus when examined with an electron microscope. The art of reconstitution has improved drastically since the phenomenon was first reported and preparations are now obtained that have 30 to 100 per cent of the specific infectivity of intact virus preparations (67). Although polymerizing protein fragments around viral nucleic acid increases specific infectivity, the reconstituted preparations still differ from intact

virus in such properties as sensitivity to inactivation by ultraviolet light and ease of dissociation into protein and nucleic acid (54, 65).

Since the nucleic acid by itself is sufficient for virus activity, the question arises as to what role the protein plays in the infection process. The current evidence suggests that the protein protects the RNA from becoming degraded and facilitates its introduction into the host cell. Since only the RNA determines the specificity of the strain of virus, including the specific TMV protein (68), the conclusion seems inescapable that the protein plays no genetic role in the reproduction of the virus. However, RNA preparations completely devoid of protein have not been obtained (69), a fact which leaves open the possibility that one, or a few, subunits still attached to the RNA as it enters a host cell may be involved in some unknown fashion with TMV replication. Claims have been made that protein contributes to the specificity of the TMV infection (64) and that preparations consisting of viral protein polymerized with DNA extracted from healthy tobacco plants are infective (70). These claims have not received independent confirmation.

It is interesting to note that although RNA is now commonly accepted as constituting the genetic material of TMV, an early piece of work seemed to contraindicate that this is so. Hollaender & Duggar (71) determined that the ultraviolet action spectrum for the inactivation of TMV departed markedly from the absorption spectrum of nucleic acid. Their result has been confirmed by more recent work which indicates that the inactivation of TMV by ultraviolet is complex and that in some strains a nucleic acid-protein interaction exists in the presence of water so as to impart considerable protection to the nucleic acid from ultraviolet damage (54, 72, 73). The virus appears to be inactivated at wavelengths of 250 mμ and longer, primarily as a result of absorption of energy by its nucleic acid. However, at shorter wavelengths the virus is inactivated primarily as a result of energy absorption by the protein moiety (74). Whether the damage arising from energy absorption by protein at 226 mμ results in the inactivation of the nucleic acid, or whether the damage is confined to the protein, is still unknown. It is possible that intact TMV is inactivated while its nucleic acid remains unharmed since Rappaport (75) has demonstrated that infectious nucleic acid can be extracted from antibody inactivated virus. The same type of phenomenon has been demonstrated for Murray Valley encephalitis virus from which infectious nucleic acid had been extracted after the virus had been inactivated with phenol (9 per cent), heat, and low pH (76).

Mutation of TMV

Strains.—Tobacco mosaic virus is a complex of many strains which are distinguished from each other by the particular symptoms they induce in host plants and by unique chemical and physical properties of the *in vitro* virus. The small size of viruses suggests that their genetic material may be

relatively uncomplicated. On this account, much interest has been attached to the study of TMV strain variation as a method of studying the chemical and physical changes underlying mutation.

Several attempts have been made to induce the appearance of variants artificially. When tobacco plants are infected with common TMV and maintained at elevated temperatures, variant strains producing milder symptoms than the parent strain are obtained (77 to 79). The high temperatures decrease the ability of the parent strain to multiply in the host tissue so it seems clear that the variant strains recovered are selected. Whether or not part of the ability to recover the variants results from an increased mutation rate at high temperatures remains an unresolved issue. A recent quantitative study led Mundry (79) to conclude that high temperature probably does increase the mutation rate of the virus.

X-irradiation of TMV has been reported to induce mutation (80), but further analysis (81) indicates the original report to be in error.

Not long after TMV was isolated as a crystalline nucleoprotein, Miller & Stanley (81a, 81b) conceived and tested the notion that the formation of variant strains of TMV might result from the chemical alteration of the virus nucleoprotein. They discovered numerous ways to alter the chemical composition of the virus protein without destroying the infectivity of the virus, but in general failed to detect the occurrence of an increased frequency of mutants. A more profitable approach to the problem of mutation has been the recent attack on the nucleic acid moiety of the virus.

Mutation of TMV with nitrous acid treatment.—Chemical modification of TMV RNA, resulting in mutation, has recently been accomplished by Mundry & Gierer (82, 83). Their work followed closely on the demonstration by Schuster & Schramm (84) that the purine and pyrimidine bases of RNA could be deaminated by nitrous acid without disrupting the polynucleotide chain. The deamination treatment results in three alterations of the nucleic acid bases: adenine is converted to hypoxanthine, guanine to xanthine, and cytosine to uracil. Hypoxanthine and xanthine are not found in untreated TMV nucleic acid, whereas uracil is a natural constituent of TMV RNA. Schuster & Schramm further determined that the degree of inactivation of the nucleic acid is an exponential function of the time of treatment with nitrous acid, demonstrating that inactivation of a nucleic acid molecule results from the deamination of a single nucleotide. Their data further suggest that deamination of any one of approximately half of the 6000 nucleotides present in the nucleic acid molecule results in inactivation. This finding led Mundry & Gierer to the notion that some particles of TMV RNA could sustain deamination without suffering inactivation and that the change in chemical composition of such deaminated, but infectious, virus might result in a change in biological properties. Their prediction was verified by finding a great variety of new strains of TMV among the survivors of a population of virus particles that had been treated with nitrous acid. Their elegant analysis of the mechanism of nitrous acid-

induced mutation of TMV has revealed that deamination of a single base is sufficient to yield a mutation. They also demonstrated that when the virus is treated so that 99.5 per cent of the infective virus particles are inactivated, about 80 per cent of the survivors of the deamination treatment produce altered symptoms in tobacco and are therefore different strains, or mutations, of TMV. Their quantitative data show that for each mutating deamination, approximately 3.5 killing deaminations occur.

Confirmation and extension of the nitrous acid experiments (85, 86) indicates that the ratio of killing to mutation is closer to 2.5 to 1 because an additional number of mutants are found by testing the surviving virus on alternate hosts. A substantial number of mutants that would otherwise have escaped detection are revealed by the smaller lesions they produce on a local lesion host. It is tempting to interpret these data to signify that deamination of guanine or adenine results in killing the virus, whereas deamination of cytosine results in mutation, although there is no evidence to indicate whether or not a virus particle containing xanthine or hypoxanthine can be infectious.

As already pointed out, Schuster & Schramm (84) have calculated that deamination of any one of approximately half of the nucleotides results in inactivation of the virus. Since only ¾ of the nucleotides are subject to deamination, it might be expected that a maximum ratio of one mutating event to two killing events would be observed on nitrous acid treatment if every deamination results in either killing or mutation. The fact that a ratio so close to this has been observed would support the notion that every base in the nucleic acid chain plays a role in determining its specificity. The fact that an almost theoretical frequency of mutation has been observed is somewhat surprising for it can be argued that most mutations might remain unrecognized because of lack of techniques for their detection. Thus, if the future reveals an appreciably higher frequency of mutation as a result of more refined observational technique, then the implied assumption that nitrous acid treatment deaminates every base in the chain with equal efficiency may be incorrect. The data of Schuster & Schramm (84) are of such a nature, however, as to suggest that the bases are all equally susceptible to deamination.

Nitrous acid-induced mutants of TMV do not show the wide range of differences found in naturally occurring strains of TMV. Natural strains may differ in a wide variety of properties, most of the differences being attributed to differences in the protein part of the virus particle. Some of the characteristics that serve to differentiate natural strains are electrophoretic mobility (87, 88), isoelectric point (89), serological properties (90, 91), amino acid composition (92), sensitivity to inactivation with ultraviolet light (93), ultraviolet absorption spectrum (94), tendency to bind a host nucleoprotein (95), and density (96). Different strains can be placed in the same group on the basis of sharing these properties although each strain produces a different symptom pattern in turkish tobacco. So far,

analyses of strains derived from nitrous acid treatment have not given evidence that mutation of the virus extends to an alteration of the protein to an extent detectable by an altered serological behavior or a change in isoelectric properties (85).

Nitrous acid treatment also induces the mutation of T2 bacteriophage (97), although with a much lower efficiency than for TMV. Since this phage contains DNA which is composed of adenine, guanine, 5-hydroxymethylcytosine, and thymine, it appears that nitrous acid can cause mutations by other mechanisms than the simple cytosine-to-uracil model proposed for TMV. It is possible that the phage DNA may contain a small amount of as yet undetected 5-methylcytosine, which upon deamination, would be converted to thymine.

RNA composition of TMV strains.—Since it has been clearly demonstrated that the nucleic acid constitutes the genetic material of the virus, a comparison of the nucleic acids of different TMV strains is of interest for the light it may shed on the nature of genetic specificity. The molar ratio of the four nucleotides found in the RNA of TMV is invariant for 16 strains of the virus (46, 98). Evidently, the genetic specificity is to be found in the arrangement of the nucleotides within the RNA chain, rather than in differing amounts of the bases. Reddi (48, 49, 51) has compared the products of pancreatic ribonuclease digestion of the isolated nucleic acid of several strains of TMV. This enzyme, as discussed earlier, will split pyrimidine-5′ phosphate linkages specifically. Reddi's results are surprising because, whereas a digest of the nucleic acid of the common strain contains 51 per cent of the total uridylic acid and 46 per cent of the total cytidylic acid residues as monopyrimidine nucleotides, a digest of the masked strain contains 71 per cent of the uridylic acid residues and 61 per cent of the cytidylic acid residues as monopyrimidine nucleotides. Thus, in the intact nucleic acid of the common strain roughly 50 per cent of the pyrimidine residues must have been preceded by a purine residue, whereas in nucleic acid of the masked strain this value drops to 35 per cent. This difference is puzzling because the common and masked strains are otherwise identical in their *in vitro* properties and can only be distinguished on the basis of their biological properties. The proteins of these two strains not only have identical amino acid compositions, but also present identical peptide maps following tryptic digestion (39). In contrast, strains such as ribgrass and yellow aucuba, whose proteins are clearly different from that of the common strain (92), have nucleic acids that yield approximately the same proportion of monopyrimidine nucleotides as does the common strain on ribonuclease digestion. Reddi (51) has also found that the nucleic acids of different strains differ in the frequency with which certain di- and tri-nucleotide sequences occur.

A confirmation of Reddi's experiments is eagerly awaited because his data indicate that a large part of the virus nucleic acid may play no role in determining protein specificity. Another feature of interest brought out

by his data is the peculiar coincidence that although the nucleic acids of different strains may differ drastically in nucleotide sequence, nevertheless, as previously mentioned, all of the strains so far examined proved to have the same nucleotide composition.

The RNA code.—It becomes clear from the foregoing discussion that infectious RNA not only contains information to control its own replication, but also contains a code that determines the production of specific viral protein. Several investigators (99) who have concerned themselves with nucleic acid coding have considered it likely that a sequence of three or four nucleotides may determine the position of a particular amino acid in a polypeptide sequence. The RNA molecule contains 6000 nucleotides, whereas the protein subunit contains 164 amino acids. Thus, there are approximately 33 nucleotides for each of the amino acids. If the RNA code does conform to the 3 to 4 nucleotides per amino acid notion, then it again becomes clear that a large part of the nucleic acid may not be concerned with determining protein specificity.

Gierer (4) has pointed out the possibility, however, that the code for each amino acid may be of the order of 30 to 40 nucleotides. This is an interesting notion because this size matches the size of "transfer" RNA (100) which transports individual amino acids to the microsome template. Possibly an accurate fitting of transfer and code RNA takes place in the positioning of an amino acid. If one of the *ca.* 33 nucleotides governing the positioning of an amino acid were altered, as is done with nitrous acid treatment, this might impair the "fit" without necessarily changing the coded amino acid. It is easy to imagine that the rate of protein synthesis might be limited by a slow programming of one amino acid, and thus, an alteration of one nucleotide might lead to the appearance of virus with unaltered protein but characterized by slow biosynthesis and reduced symptom expression. Such a scheme may account for the many small lesion mutants which appear on nitrous acid treatment.

The Intracellular Behavior of TMV

Although spectacular progress has been made in unraveling the chemistry and structure of TMV and the minimum configuration essential for infectivity, progress in describing the behavior of TMV while it is in the process of infection has been slow. It is probable, however, that only through an understanding of the intracellular events that lead to production of virus, will insight be gained into the basic biological problems of the replication of RNA and the means by which the presence of viral RNA leads to the production of strain-specific viral protein. One of the chief difficulties in the study of TMV infection is the lack of efficient techniques for single-cell culture of host cells. Several recent reports (101 to 104), however, give promise that this difficulty may soon be overcome.

Current knowledge of the intracellular behavior of TMV will be considered in terms of the following aspects of the infective process: (*a*) the

initial act of infection of the host cells; (*b*) the behavior of virus after infection but prior to multiplication; (*c*) the time and manner of multiplication; and (*d*) the spread of virus from initial infected cells to adjacent cells.

The initial act of infection.—Before infection of a plant leaf by TMV can occur, the surface of the host leaf must be abraded. Without abrasion, no infection will occur even if the intracellular spaces of the host leaf are filled with high concentrations of virus (105). Yarwood (106) has recently reviewed the different techniques commonly employed for abrasion. The process of abrasion can be thought of as creating "infectible sites" on the leaf surface where infection can occur if virus particles are present. Although the physical nature of the infectible sites is completely unknown at present, several of their properties have been explored.

The physiology of the host at the time of abrasion plays a large part in determining the number of infectible sites that will be produced. Factors such as time of day, nutrition, pre-exposure of the host to light or dark, and different temperatures can markedly affect the number of infectible sites produced by abrasion (107 to 109). The infectible sites do not appear to be distributed randomly over the surface of the leaf (110), but there is no evidence at the present time to associate them with any of the specialized structures of the leaf surface such as leaf hairs (111). Abrasion, although creating infectible sites, lowers the ability of a subsequent abrasion to produce infectible sites and also destroys recently infected sites (112, 113). Lindner *et al.* (113) have interpreted data obtained when cucumber cotyledons were abraded several times in succession as signifying that when a leaf is abraded, epidermal cells are either not injured enough, injured too much, or injured properly. They consider that those cells which have been "injured properly" constitute infectible sites.

There is some question as to whether or not all infectible sites are equally susceptible to infection. Kleczkowski (114) concludes that the susceptibility of the sites to infection is distributed according to the logarithm of a normal distribution. The data of others, however, appear to be compatible with the notion that the infectible sites are equally susceptible (115 to 117).

The infectible sites, once created, disappear rapidly in a matter of seconds to minutes (118, 119). The rate of loss of the sites depends on the physiological state of the leaf. For instance, the loss is slowed in potassium-deficient plants (120). The rate of loss is also different for sites produced on different hosts (121).

Establishment of the infective center.—When an infectible site is placed in contact with infectious particles, the site becomes infected and an infective center is created. Immediately upon the creation of an infective center, the infecting particle becomes firmly attached to the leaf in such a manner that it cannot be removed by vigorous washing of the leaf with water (3, 123). The infecting particle also becomes resistant to reagents that

would inactivate it when free in solution. For instance, if nucleic acid is used as inoculum, infective centers are not destroyed by dipping inoculated leaves into strong ribonuclease solutions immediately after inoculation (124). The evidence is fairly good, therefore, that the infectious entity penetrates directly into a cell where it becomes resistant to agents which would inactivate it in the extracellular state.

Most investigators find that a minimum of 10^6 to 10^7 TMV particles must be applied to the leaf for each infective center formed. There have been reports of infective center induction with fewer particles (125, 126), but these reports have not been confirmed (127). This extremely low "plating efficiency" probably results from two causes: (*a*) a low probability that an infective particle will come into contact with one of the limited number of infectible sites produced by abrasion, and (*b*) the bulk of the particles present in a TMV preparation are non-infectious (128, 129).

Evidence that implies that a single virus particle is sufficient to convert an infectible site into an infective center is found in the relationship between concentration of virus applied to the leaf and number of local lesions engendered. An analysis of the data based on a Poisson distribution suggests that infective centers are formed as the result of chance encounters between single virus particles and infectible sites (114 to 116). Because of the rapid loss of infectible sites after their creation, maximum numbers of infective centers are produced only when the virus is present at the instant the leaf is abraded. Abrading a *Nicotiana glutinosa* leaf and then dipping the leaf into inoculum as quickly as possible, will result in a 50 to 75 per cent decrease in the number of infective centers, when compared with results of abrasion in the presence of the virus.

An interesting feature of the infective process is an exclusion phenomenon that appears to allow one, and only one, virus particle to accomplish the conversion of the infectible site to an infective center. Two types of evidence reveal this phenomenon (130, 131). One type is derived from inactivation of the infective centers by ultraviolet irradiation [see Latarjet (132) for interpretation of radiation inactivation data]. Inactivation of infective centers was found to be an exponential function of the dose of radiation over a wide range of concentrations of virus applied as inocula. With the higher concentrations, it was calculated that each site had been presented with several virus particles, each capable of converting the site to an infective center. Had all of the particles present at each infectible site participated in establishing the infective center, a multi-target type of inactivation curve would have been found.

The second type of evidence for an exclusion phenomenon is derived from a study of competition between two strains of TMV applied to a differential host. The host is differential in the sense that it can be infected by both strains but only one of the strains will cause the formation of local lesions, the other producing systemic symptoms instead. When mixtures of the two strains are applied in different ratios, fewer local lesions arise than

when the lesion-producing strain is applied alone at the same concentration. The quantitative data suggest that even though the statistical opportunity was presented for the site to be entered by particles of both strains, only one or the other, but not both, converted the site to an infective center.

The conclusion that a maximum of one infectious particle can participate in an infection can be used as evidence that the infectible site has a specific mechanism whereby only one virus particle is permitted to multiply. Other evidence for the specificity of virus-infectible site interaction is the observation that virus inactivated by ultraviolet light or antiserum does not compete for infectible sites with untreated virus (128), except in the case of very high concentrations of ultraviolet inactivated virus (133). Evidently, subtle changes that may affect either the protein or nucleic acid are sufficient to render a virus particle incapable of participating in even the earliest events of infection, perhaps by altering its ability to make proper contact with the infectible site.

Although infection is generally initiated by abrading the host leaf, several investigators have reported limited success in initiating infection by cutting or puncturing single leaf hairs in the presence of virus (118, 134, 135). The efficiency of infection by this method is low. Benda (135) has reported that 30 per cent of his treated leaf hairs became infected, but this high value was obtained only when the hairs were exposed to extremely high concentrations of virus. In contrast to the exclusion phenomenon observed when inoculation is performed by the abrasion method, it is possible to obtain infective centers containing two strains of virus when leaf hairs are inoculated (136).

The early events of infection.—After establishment of the infective center, one of two kinds of reaction will subsequently occur, depending upon the type of host. With hosts such as *N. tabacum,* the infective centers are not conspicuous. The virus spreads throughout the inoculated leaf and also invades other tissues of the plant. Infective centers can be revealed, however, by staining for starch accumulations (137). In other hosts, such as *N. glutinosa* and *N. tabacum* var. Xanthi (138), a group of cells which have sustained virus infection, surrounding the infective center, suddenly collapse to form a necrotic local lesion. The lesion continues to increase in size as virus continues to invade surrounding leaf cells. Generally, the virus does not escape from these local areas to invade other parts of the plant. Whether or not the reaction is necrotic or systemic is a characteristic of the particular virus strain-host combination. *N. sylvestris,* for example, responds to some strains of TMV by the local necrotic lesion reaction, whereas other strains produce the systemic reaction.

Little is known of the nature of the infective center soon after it is established. It seems reasonable to presume, however, that it consists of a single cell in association with a virus particle. Several studies indicate that infective centers pass through several critical stages in their develop-

ment. For instance, Yarwood (139) has found that a heat treatment applied to bean leaves 5 hr. after inoculation with TMV results in a fivefold increase in the number of local lesions that appear. This datum can be interpreted as signifying that many of the infective centers initiated at the time of inoculation die unless they are rescued by the heat treatment at a critical period in their history. It has also been reported that the infective centers on *N. glutinosa* are sensitive to darkness for the first 30 min. after their establishment (140). Still other effects of light and temperature on the development of infective centers have recently been reviewed by Bawden (2).

A roadblock to the study of the intracellular events of plant virus infection exists in the fact that initially it is possible to infect only about 10^3 of the approximately 10^8 cells of the leaf. It is desirable to study these initially infected cells because after the virus has spread to other cells of the leaf, experiments become confounded by the presence of cells in all different stages of infection and it becomes difficult to define the sequence of infective events as they presumably occur in each individual cell. Although methods are presently unavailable for the detection and isolation of the initially infected cells, nevertheless, techniques have been developd for their study. One such technique is based on the changes of sensitivity to various agents of infective centers while they are still single cells.

Inactivation of infective centers by ultraviolet light has proven to be a powerful tool for studying the changes of the infectious principle in the early stages of infection (112, 130, 141). When leaves of a local lesion host are irradiated within a short period of time after they have been inoculated, fewer lesions will arise than on unirradiated control leaves. The loss of infective centers results from the inactivation of the infecting virus particle. When the experiments are carried out under constant conditions of light and temperature, it is observed that infective centers induced by a particular strain (U2) do not change in sensitivity to irradiation by ultraviolet for a period of 2 hr. With another strain, U1, the first change in ultraviolet sensitivity is apparent only after a much longer period of time. In sharp contrast to the behavior of intact TMV used as inoculum, the infective centers induced by TMV nucleic acid decrease in sensitivity almost immediately after their establishment. These results have been interpreted as signifying that the first 2 hr. of infection with intact virus particles involves the preparation of virus for further events of infection, a preparation which is not required when nucleic acid is used as inoculum. With intact virus, the first events can be visualized as a stripping of the protein subunits from the nucleic acid. The observation that certain events of infection are by-passed when nucleic acid is used as inoculum finds further support from experiments that show that lesions induced by TMV nucleic acid appear sooner than lesions induced by intact virus (142, 143). Evidently, the events that are by-passed in the first cells to be infected can still be recognized even after the virus has spread through hundreds of cells surrounding the

infective centers. It has also been found that TMV infectious material can be extracted sooner from leaves inoculated with nucleic acid than with intact virus (126, 127). Hamers-Casterman & Jeener (144) have observed that infective centers can be inactivated by ribonuclease if this enzyme is infiltrated into leaves within 2 hr. after inoculation. This may indicate that the viral nucleic acid becomes exposed during this period and subject to enzymatic degradation. [Extracellular intact virus is not irreversibly degraded by ribonuclease (145).] It is not clear, however, whether the enzyme acts to destroy the infectious principle or a host cell component necessary to maintain the early infection.

There is evidence to indicate that nucleic acid released from viral protein within the infective center does not exist in the same "free" state as nucleic acid arriving directly from the inoculum to create the infective center. TMV strain U1 is 5.5 times more resistant to inactivation with ultraviolet than its extracted nucleic acid (54, 72). The U1 infective center has the same sensitivity to inactivation as the *in vitro* U1 virus and does not increase in sensitivity as would be expected if the nucleic acid released from the virus within the infective center were in the same state as extracellular infectious RNA (130). It is possible that the nucleic acid, upon release from protein, still does not pass through a "free" state before multiplying, but rather remains protected from radiation damage by association with a host material. A similar notion has been postulated to account for radiation resistance during the early events of infection of bacteria with T—even bacteriophages (146).

Another indication that nucleic acid from an infecting virus particle does not become "free" is found in the work of Bawden & Kleczkowski (147, 148). They found that infective centers initiated with ultraviolet-damaged infectious nucleic acid were subject to photoreactivation, whereas the phenomenon was absent when infection was initiated with intact virus. Since light reactivation of viruses generally must take place with the cooperation of the host cell (149), it would seem that the nucleic acid of the intact virus does not enter a state where it can interact with the appropriate host components to be photoreactivated. This interpretation is somewhat obscured, however, because photoreactivation is not observed with nucleic acid that has been extracted from irradiated virus.

With intact U2 TMV serving as inoculum, as many as four phases in the radiation sensitivity of the infective centers have been detected. The first three phases are characterized by exponential survival curves of the infective centers. The fact that the survival curves are exponential during this period of time (*ca.* 6 hr.) is taken as evidence that there has been no increase in the number of units independently capable of maintaining the infection; that is, that virus multiplication has not begun.

Between 6 and 7 hr. after the initiation of infection with intact virus, the fourth phase is encountered, characterized by a shift in the character of the radiation survival curves of the infective centers from exponential

to multi-target. This is interpreted to mean that several units, each of which is independently capable of maintaining the infection, are now present in each infective center. The nature of these units is unknown and can only be elucidated by further experimentation. It seems probable, however, that these units are not intact virus particles, as they are known in the extracellular state. Evidence for this notion is supplied by the behavior of infective centers induced by TMV nucleic acids. In this case, newly formed infectious units are detected approximately 3 hr. after inoculation. No difference in the radiation sensitivity of the infective centers was detectable regardless of whether U1 or U2 nucleic acid was used for inoculation. The behavior of nucleic acid infective centers is thus in marked contrast to centers initiated by intact U1 and U2 virus, the latter differing by more than five times in their sensitivity to inactivation by ultraviolet. It seems, therefore, that the first new units to arise from nucleic acid infections do not display the extreme divergence in radiation sensitivity that would be expected if the new units were intact virus particles.

It is possible that each cell can have only one center responsible for virus production and that this indeed is the basis for the exclusion phenomenon previously noted. In this case one might speculate that the new units independently capable of maintaining the infection actually represent newly infected cells. This possibility can only be answered when definitive experiments are performed to find out how long the initially infected cell of an infective center is the only cell infected and this information is coordinated with the data from the radiation experiments.

Actually, no studies have as yet been made to find out how long it takes for the TMV infection to spread from an initially infected cell to adjacent cells. Such a study has been made with cucumber mosaic virus on the host cowpea. By stripping the abraded upper epidermis from the leaf at different times after inoculation, Welkie & Pound (150) were able to determine that infection had been established in tissue below the epidermis within approximately 8 hr. after infection when the leaves were incubated at 20°C. The time for reinfection was different at other temperatures. There is no reason to suspect that TMV behaves in a qualitatively different manner.

Several efforts have been made to determine how soon after inoculation newly synthesized virus appears in the leaf. The investigations have the common feature of inoculating leaf tissue with TMV and then extracting the tissue at different times after inoculation. These experiments are difficult to perform and difficult to interpret for the following reasons: (*a*) It is possible to infect only about 10^{-5} of the cells at the time of inoculation. Thus, the virus must be extracted from a great excess of non-infected leaf tissue. (*b*) The most sensitive method for detecting the presence of virus, that of bioassay, is inefficient, so that newly synthesized virus may be present in infected cells before it can be detected. (*c*) Under many circumstances infectious particles that have not participated in infection adhere to the

leaf, are recovered during the extraction procedure, and serve to confound the results. The studies that have been performed, however, serve to indicate a maximum time period after inoculation during which newly synthesized virus has not yet appeared. Yarwood (151) detected virus in tobacco leaves held at 31°C. between 8 and 10 hr. after inoculation. Kassanis (127) was able to confirm these observations and, in addition, noted that newly synthesized virus could be detected 2 hr. sooner from leaves inoculated with nucleic acid than from leaves inoculated with intact virus. Other workers have been able to detect virus only after longer periods of time (126, 152, 153), but whether their efficiency of assay was lower or whether they worked with plants under different physiological conditions cannot be assessed.

Although little is known about the mechanism by which the infected cell produces new virus particles, recent experimental data can be used to argue that the two components of the virus, nucleic acid and protein, are produced separately, and that production of intact virus represents a final assembly process. Cochrane & Chidester (154) have demonstrated that free, infectious nucleic acid can be extracted from TMV-infected plants. Engler & Schramm (155), moreover, have shown that in very early infections, free nucleic acid comprises the bulk of the total extractable infectious material. As the infection proceeds, however, the ratio of free infectious nucleic acid to intact virus in leaf extracts drops markedly. It would seem, therefore, that RNA replication precedes viral protein synthesis or, at any rate, that RNA replication proceeds at a much more rapid rate than protein synthesis in the early infection. Just as it is possible to extract proteinless infectious nucleic acid from infected tissue, so has it proved possible to recover nucleic acid-free protein particles that resemble the viral protein subunits which can be prepared by degrading the virus (156 to 160). These protein particles can be aggregated into the cylindrical shape of TMV *in vitro* (156, 157, 161) and two groups of workers (162, 163) have obtained evidence to indicate that these nucleic acid-free protein particles are precursors of viral protein.

Speculations on virus synthesis.—There is now such a wealth of information available on virus behavior, protein and nucleic acid synthesis, and specific biochemical functions of cellular components that it is possible to integrate some of these findings in the form of speculations concerning virus behavior within the host cell.

Evidence obtained from several systems indicates that much of the RNA found in the cytoplasm of a cell is synthesized in the nucleus (164 to 166). We suggest, therefore, that viral RNA is also synthesized in the nucleus. Circumstantial evidence for this notion is supplied by the work of Zech & Vogt-Kohne (167), who noted that among the earliest cytological signs of TMV infection in a hair cell are: first an increase, and then a decrease in the nucleic acid contained in the nucleus. Concomitant with the decrease of nucleic acid in the nucleus, they observed an increase of this material in the cytoplasm. The manner in which the viral RNA self-repli-

cates is unknown but it is conceivable that it occurs by means of an obligate, alternate transfer of specificity between DNA and RNA, according to the model recently proposed by Stent (146).

Recent biochemical evidence has indicated that the seat of intracellular protein synthesis lies in those structures that are extractable from cells as microsomes (168, 169). Some microsomal particles have been shown to contain RNA of an amount and size similar to that contained in intact TMV particles (170, 171). We postulate, therefore, that as newly synthesized viral RNA leaves the nucleus, it combines with microsomal protein to form a new class of microsomes which governs the synthesis of TMV protein subunits.

The assembly of the virus nucleoprotein particle may now be visualized as polymerization of protein subunits produced by "TMV microsomes" around newly produced RNA as it escapes from the nucleus. We presume that the nucleus continues to synthesize host nucleic acid as well as TMV RNA. It is possible that these two different nucleic acids are so much alike that both are susceptible to being surrounded by the TMV protein subunits. The result of this confusion is that many TMV particles will contain noninfectious, host nucleic acid but will otherwise be indistinguishable from infectious particles when extracted from the cell. The extracellular virus preparation will, as a consequence, have a low specific infectivity, which is what has been observed (128). There is some evidence available to indicate that the chloroplast may be the site of assembly of the virus from its constituent parts (163, 172).

Transfer of TMV from cell to cell.—Having presented a view of virus activity in the first cells of a leaf to become infected, the rate and mode by which the infection spreads from the first cells to adjacent cells remains to be considered. Uppal (173) measured the time required for the TMV infection to traverse the distance separating the upper from the lower epidermis of leaves. From an estimate of the number of cells that the virus must have passed through to reach the lower epidermis, he arrived at a rate of transfer equivalent to about one cell per 4 to 6 hr. This rate of transfer from cell to cell is nearly the same as found by measuring the growth of lesions on another host (174). The radius of a local lesion increases linearly with time, and the rate of increase is a characteristic of the TMV strain. With the U1 strain, the rate of spread of necrotic cells was found to be one cell per 4 hr. along a radius. With the U2 strain, the rate was one cell per 6 hr. Microscopic examination of sections through lesions indicated the virus to have invaded approximately four cells beyond those that had already become necrotic. The estimate was made, therefore, that a cell becomes necrotic about 16 hr. after having been invaded by TMV. Infectivity measurements showed the average necrotic cell to contain between 10^4 and 10^5 virus particles which were manufactured during a period 16 to 20 hr. The route by which the virus is transferred from cell to cell is thought to be the plasmodesmatal connections (175, 176). Whether the infectivity is transferred as nucleic acid, or as virus nucleoprotein is unknown.

Quantitative lesion-appearance studies (143) have thrown some light on the process of necrosis of infected cells because it was found that fewer cells need to collapse for the sudden appearance of a lesion with the U2 strain than with the U1 strain. Furthermore, the studies show that from the start, the formation of a lesion is not a continuous process whereby the first cell to be infected is the first to collapse, followed in succession by collapse of adjacent cells until the number is sufficient to make the lesion visible. Rather, lesions appear when a group of about 200 cells surrounding the infective center suddenly collapse.

Transfer of TMV through cells of the vascular system.—This subject was reviewed by Bennett in Volume 7 of this series (1). Since then, evidence has been presented to show that there is a restriction of the systemic spread of TMV (177). When single, mature leaves are inoculated with a mixture of two strains of TMV, frequently one of the two strains will not be found in the top of the plant even though both strains multiplied to a large extent in the inoculated leaf. The frequency of exclusion of one of the two strains was found to be a function of the ratio of the two strains applied in the inoculum. An hypothesis was advanced that exclusion arises because only a few of the infective centers on the inoculated leaf can contribute virus for the systemic invasion, or that only a very few virus particles institute the systemic infection.

LITERATURE CITED

1. Bennett, C. W., *Ann. Rev. Plant Physiol.*, **7**, 143 (1956)
2. Bawden, F. C., *Ann. Rev. Plant Physiol.*, **10**, 239 (1959)
3. Wildman, S. G., in *The Viruses*, **2** (Academic Press, New York, N.Y., 1959)
4. Gierer, A., *Progr. in Biophys. and Biophys. Cytol.*, **10** (In press)
5. Porter, C. A., *Advances in Virus Research*, **6**, 75 (1959)
6. Schramm, G., and Bergold, G., *Z. Naturforsch.*, **2b**, 108 (1947)
7. Boedtker, H., and Simmons, N. S., *J. Am. Chem. Soc.*, **80**, 2550 (1958)
8. Williams, R. C., and Steere, R. L., *J. Am. Chem. Soc.*, **73**, 2057 (1951)
9. Schramm, G., and Wiedemann, M., *Z. Naturforsch.*, **6b**, 379 (1951)
10. Hall, C. E., *J. Am. Chem. Soc.*, **80**, 2556 (1958)
11. Steere, R. L., *J. Biophys. Biochem. Cytol.*, **3**, 57 (1957)
12. Franklin, R. E., and Klug, A., *Biochim. et Biophys. Acta*, **19**, 403 (1956)
13. Knight, C. A., and Woody, B. R., *Arch. Biochem. Biophys.*, **78**, 460 (1958)
14. Loring, H. S., and Waritz, R. S., *Science*, **125**, 646 (1957)
15. Loring, H. S., Al-Rawi, S., and Fujumoto, Y., *J. Biol. Chem.*, **233**, 1415 (1958)
16. Harris, J. L., and Knight, C. A., *J. Biol. Chem.*, **214**, 215 (1955)
17. Franklin, R. E., and Holmes, K. C., *Acta Cryst.*, **11**, 213 (1958)
18. Watson, J. D., *Biochim. et Biophys. Acta*, **13**, 10 (1954)
19. Franklin, R. E., and Klug, A., *Acta Cryst.*, **8**, 777 (1955)
20. Gierer, A., *Nature*, **179**, 1297 (1957)
21. Gierer, A., *Z. Naturforsch.*, **13b**, 477 (1958)
22. Ginoza, W., *Nature*, **181**, 958 (1958)
23. Staehelin, M., *Biochim. et Biophys. Acta*, **29**, 410 (1958)
24. Casper, D. L. D., *Nature*, **177**, 928 (1956)

25. Franklin, R. E., *Nature*, **177**, 929 (1956)
26. Ansevin, A. T., and Lauffer, M. A., *Nature*, **183**, 1601 (1959)
27. Anderer, F. A., *Z. Naturforsch.*, **14b**, 24 (1959)
28. Wittmann, H. G., *Experientia*, **15**, 174 (1959)
29. Ramachandran, L. K., *Virology*, **5**, 244 (1958)
30. Aach, H. G., *Z. Naturforsch.*, **13b**, 425 (1958)
31. Ramachandran, L. K., and Narita, K., *Biochim. et Biophys. Acta*, **30**, 616 (1958)
32. Harris, J. L., and Knight, C. A., *Nature*, **170**, 613 (1952)
33. Niu, C.-I., and Fraenkel- Conrat, H., *Biochim. et Biophys. Acta*, **16**, 597 (1955)
34. Braunitzer, G., *Naturwissenschaften*, **12**, 371 (1955)
35. Niu, C.-I., and Fraenkel-Conrat, H., *Arch. Biochem. Biophys.*, **59**, 538 (1955)
36. Narita, K., *Biochim. et Biophys. Acta*, **28**, 184 (1958)
37. Anderer, F. A., *Z. Naturforsch.*, **14b**, 363 (1959)
38. Gish, D. T., Ramachandran, L. K., and Stanley, W. M., *Arch. Biochem. Biophys.*, **78**, 433 (1958)
39. Woody, B. R., and Knight, C. A., *Virology* (In press)
40. Schramm, G., and Braunitzer, G., *Naturwissenschaften*, **42**, 74 (1955)
41. Ramachandran, L. K., and Gish, D. T., *J. Am. Chem. Soc.*, **81**, 884 (1959)
42. Ingraham, V. M., *Nature*, **178**, 792 (1956)
43. Doty, P., Boedtker, H., Fresco, J. R., Hazelkorn, R., and Litt, M., *Proc. Natl. Acad. Sci. U.S.*, **45**, 482 (1959)
44. Haschmeyer, R., Singer, B., and Fraenkel-Conrat, H., *Proc. Natl. Acad. Sci. U.S.*, **45**, 313 (1959)
45. Cheo, P. C., Friesen, B. S., and Sinsheimer, R. L., *Proc. Natl. Acad. Sci. U.S.*, **45**, 303 (1959)
46. Knight, C. A., *J. Biol. Chem.*, **197**, 241 (1952)
47. Reddi, K. K., and Knight, C. A., *J. Biol. Chem.*, **221**, 629 (1956)
48. Reddi, K. K., *Biochim. et Biophys. Acta*, **25**, 528 (1957)
49. Reddi, K. K., *Proc. Natl. Acad. Sci. U.S.*, **45**, 293 (1959)
50. Hart, R. G., *Proc. Natl. Acad. Sci. U.S.*, **43**, 57 (1957)
51. Reddi, K. K., *Biochim. et Biophys. Acta*, **32**, 386 (1959)
52. Fraenkel-Conrat, H., *J. Am. Chem. Soc.*, **78**, 882 (1956)
53. Gierer, A., and Schramm, G., *Nature*, **177**, 702 (1956)
54. Siegel, A., Ginoza, W., and Wildman, S. G., *Nature*, **178**, 1117 (1956)
55. Bawden, F. C., and Pirie, N. W., *J. Gen. Microbiol.*, **17**, 80 (1957)
56. Wecker, E., and Schaeffer, W., *Z. Naturforsch.*, **12b**, 415 (1957)
57. Alexander, H. E., Koch, G., Mountain, I. M., Sprunt, K., and Van Damme, O., *Virology*, **5**, 172 (1958)
58. Kaper, J. M., and Steere, R. L., *Virology*, **7**, 127 (1959)
59. Rushizky, G. W., and Knight, C. A., *Virology*, **7**, 448 (1959)
60. Kaper, J. M., and Steere, R. L., *Virology*, **7**, 527 (1959)
61. Ginoza, W., and Norman, A., *Nature*, **179**, 520 (1957)
62. Commoner, B., Shearer, G. B., and Strode, C., *Proc. Natl. Acad. Sci. U.S.*, **44**, 1117 (1958)
63. Commoner, B., Lippincott, J. A., Shearer, G. B., Richman, E. E., and Wu, J.-H., *Nature*, **178**, 767 (1956)
64. Commoner, B., *N. Y. Acad. Sci., Special Pub.*, No. 5, 237 (1957)
65. Fraenkel-Conrat, H., and Williams, R. C., *Proc. Natl. Acad. Sci. U.S.*, **41**, 690 (1955)

66. Lippincott, J. A., and Commoner, B., *Biochim. et Biophys. Acta,* **19,** 198 (1956)
67. Fraenkel-Conrat, H., *Biochim. et Biophys. Acta,* **33,** 359 (1959)
68. Fraenkel-Conrat, H., and Singer, B., *Biochim. et Biophys. Acta,* **24,** 540 (1957)
69. Ramachandran, L. K., and Fraenkel-Conrat, H., *Arch. Biochem. Biophys.,* **74,** 224 (1958)
70. Wang, T. Y., and Commoner, B., *Proc. Natl. Acad. Sci. U.S.,* **42,** 831 (1956)
71. Hollaender, A., and Duggar, B. M., *Proc. Natl. Acad. Sci. U.S.,* **22,** 19 (1936)
72. McLaren, A. D., and Takahashi, W. N., *Radiation Research,* **6,** 532 (1947)
73. Siegel, A., *Nature,* **180,** 1430 (1957)
74. Siegel, A., and Norman, A., *Virology,* **6,** 725 (1958)
75. Rappaport, I., *Nature* (In press)
76. Ada, G. L., and Anderson, S. G., *Nature,* **183,** 799 (1959)
77. Johnson, J., *Science,* **64,** 210 (1925)
78. Holmes, F. O., *Phytopathology,* **24,** 845 (1934)
79. Mundry, K. W., *Z. Induktive Abstammungst u. Vererbungslehre,* **88,** 407 (1957)
80. Gowen, J. W., *Cold Spring Harbor Symposia Quant. Biol.,* **9,** 187 (1941)
81. Mundry, K. W., *Z. Induktive Abstammungs u. Vererbungslehre,* **88,** 115 (1957)
81a. Miller, G. L., and Stanley, W. M., *J. Biol. Chem.,* **141,** 905 (1941)
81b. Miller, G. L., and Stanley, W. M., *J. Biol. Chem.,* **146,** 331 (1944)
82. Mundry, K. W., and Gierer, A., *Z. Vererbungslehre,* **89,** 614 (1958)
83. Gierer, A., and Mundry, K. W., *Nature,* **182,** 1457 (1958)
84. Schuster, H., and Schramm, G., *Z. Naturforsch.,* **13b,** 697 (1958)
85. Siegel, A., *Genetics* (In press)
86. Siegel, A., *Phytopathology,* **49,** 550 (1959)
87. Knight, C. A., and Lauffer, M. A., *J. Biol. Chem.,* **144,** 411 (1942)
88. Friedrich-Freksa, H., Melchers, G., and Schramm, G., *Biol. Zentr.,* **65,** 187 (1946)
89. Oster, G., *J. Biol. Chem.,* **190,** 55 (1951)
90. Bawden, F. C., and Pirie, N. W., *Brit. J. Exptl. Pathol.,* **18,** 275 (1937)
91. Chester, K. S., *Phytopathology,* **76,** 778 (1936)
92. Knight, C. A., *J. Biol. Chem.,* **171,** 297 (1947)
93. Siegel, A., and Wildman, S. G., *Phytopathology,* **44,** 277 (1954)
94. Ginoza, W., and Atkinson, D. E., *Virology,* **1,** 253 (1955)
95. Ginoza, W., Atkinson, D. E., and Wildman, S. G., *Science,* **119,** 269 (1954)
96. Siegel, A., and Hudson, W., *Biochim. et Biophys, Acta,* **34,** 254 (1959)
97. Vielmetter, W., and Wieder, C. M., *Z. Naturforsch.,* **14b,** 312 (1959)
98. Black, F. L., and Knight, C. A., *J. Biol. Chem.,* **202,** 51 (1953)
99. Levinthal, C., *Revs. Modern Phys.,* **31,** 249 (1959)
100. Hoagland, M. B., Zamecnik, P. C., and Stephenson, M. L., *Biochim. et Biophys. Acta,* **24,** 215 (1957)
101. Kassanis, B., *Virology,* **4,** 5 (1957)
102. Kassanis, B., Tinsley, T. W., and Quak, F., *Ann. Appl. Biol.,* **46,** 11 (1958)
103. Hildebrandt, A. C., *Proc. Natl. Acad. Sci. U.S.,* **44,** 354 (1958)
104. Bergmann, L., *Trans. N. Y. Acad. Sci.,* Ser. 2, **21,** 227 (1959)
105. Caldwell, J., *Ann. Appl. Biol.,* **21,** 191 (1934)
106. Yarwood, C. E., *Advances in Virus Research,* **4,** 243 (1957)
107. Matthews, R. E. F., *Ann. Appl. Biol.,* **40,** 377 (1953)

108. Bawden, F. C., and Roberts, F. M., *Ann. Appl. Biol.*, **34**, 286 (1947)
109. Kassanis, B., *Ann. Appl. Biol.*, **39**, 358 (1952)
110. Rappaport, I., and Wildman, S. G., *Phytopathology*, **49**, 231 (1959)
111. Boyle, L. M., and McKinney, H. H., *Phytopathology*, **28**, 114 (1938)
112. Bawden, F. C., and Harrison, B. D., *J. Gen. Microbiol.*, **13**, 494 (1955)
113. Lindner, R. C., Kirkpatrick, H. C., and Weeks, T. E., *Phytopathology*, **49**, 78 (1959)
114. Kleczkowski, A., *J. Gen. Microbiol.*, **4**, 53 (1950)
115. Youden, W. J., Beale, H. P., and Guthrie, J. D., *Contribs. Boyce Thompson Inst.*, **7**, 37 (1935)
116. Bald, J. G., *Ann. Appl. Biol.*, **24**, 33 (1937)
117. Lauffer, M. A., and Price, W. C., *Arch. Biochem.*, **8**, 449 (1945)
118. Sheffield, F. M. L., *Ann. Appl. Biol.*, **23**, 498 (1936)
119. Allington, W. B., and Laird, E. F., Jr., *Phytopathology*, **44**, 546 (1954)
120. Allington, W. B., and Laird, E. F., Jr., *Phytopathology*, **44**, 297 (1954)
121. Jedlinski, H., *Phytopathology*, **46**, 673 (1956)
123. Holmes, F. O., *Botan. Gaz.*, **87**, 39 (1929)
124. Siegel, A., cited in *The Viruses*, **2** (Wildman, S. G., Academic Press, New York, N.Y., 1959)
125. Steere, R. L., *Phytopathology*, **45**, 196 (1955)
126. Schramm, G., and Engler, R., *Nature*, **181**, 916 (1958)
127. Kassanis, B., *J. Gen. Microbiol.*, **20**, 704 (1959)
128. Rappaport, I., and Siegel, A., *J. Immunol.*, **74**, 106 (1955)
129. Pirie, N. W., *Advances in Virus Research*, **4**, 159 (1957)
130. Siegel, A., and Wildman, S. G., *Virology*, **2**, 69 (1956)
131. Siegel, A., *Virology*, **8**, 470 (1959)
132. Latarjet, R., in *The Nature of Virus Multiplication* (Cambridge Univ. Press, New York, N.Y., 1953)
133. Bawden, F. C., and Kleczkowski, A., *J. Gen. Microbiol.*, **8**, 145 (1953)
134. Zech, H., *Planta*, **40**, 461 (1952)
135. Benda, G. T. A., *Virology*, **6**, 49 (1958)
136. Benda, G. T. A., *Virology*, **2**, 820 (1956)
137. Holmes, F. O., *Contribs. Boyce Thompson Inst.*, **3**, 163 (1931)
138. Takahashi, W. N., *Phytopathology*, **46**, 654 (1956)
139. Yarwood, C. E., *Phytopathology*, **48**, 39 (1958)
140. Goodchild, D. J., *The Effect of Light on the Reproduction of Tobacco Mosaic* (Doctoral thesis, University of Sydney, Sydney, Australia, 1958)
141. Siegel, A., Ginoza, W., and Wildman, S. G., *Virology*, **3**, 554 (1957)
142. Fraenkel-Conrat, H., Singer, B., and Veldee, S., *Biochim. et Biophys. Acta*, **29**, 639 (1958)
143. Wildman, S. G., and Ford, C. V., *Phytopathology* (In press)
144. Hamers-Casterman, C., and Jeener, R., *Virology*, **3**, 197 (1957)
145. Loring, H. S., *J. Gen. Physiol.*, **25**, 497 (1942)
146. Stent, G. S., *Advances in Virus Research*, **5**, 95 (1958)
147. Bawden, F. C., and Kleczkowski, A., *Nature*, **183**, 503 (1959)
148. Bawden, F. C., and Kleczkowski, A., *J. Gen. Microbiol.*, **13**, 370 (1955)
149. Dulbecco, R., *J. Bacteriol.*, **59**, 329 (1950)
150. Welkie, G. W., and Pound, G. S., *Virology*, **5**, 362 (1958)
151. Yarwood, C. E., *Am. J. Botany*, **39**, 613 (1952)

152. Harrison, B. D., *J. Gen. Microbiol.*, **15**, 210 (1956)
153. Kohler, E., *Arch. Mikrobiol.*, **33**, 128 (1959)
154. Cochrane, G. W., and Chidester, J. L., *Virology*, **4**, 390 (1957)
155. Engler, R., and Schramm, G., *Nature*, **183**, 1277 (1959)
156. Takahashi, W. N., and Ishii, M., *Am. J. Botany*, **40**, 85 (1953)
157. Jeener, R., and Lemoine, P., *Nature*, **171**, 935 (1953)
158. Commoner, B., Newmark, P., and Rodenberg, S. D., *Arch. Biochem. Biophys.*, **37**, 15 (1952)
159. Schramm, G., Schumacher, G., and Zillig, W., *Z. Naturforsch.*, **10b**, 481 (1955)
160. Fraenkel-Conrat, H., *Virology*, **4**, 1 (1957)
161. Rich, A., Dunitz, I. D., and Newmark, P., *Nature*, **175**, 1074 (1955)
162. Rysselberge, C. van, and Jeener, R., *Biochim. et Biophys. Acta*, **17**, 158 (1957)
163. Boardman, N. K., and Zaitlin, M., *Virology*, **6**, 758 (1958)
164. Zalokar, M., *Proc. Intern. Congr. Genetics, 10th Congr.*, **2**, 330 (1958)
165. Goldstein, L., and Plaut, W., *Proc. Natl. Acad. Sci. U. S.*, **41**, 874 (1955)
166. Marshak, A., and Calvet, F., *J. Cellular Comp. Physiol.*, **34**, 451 (1949)
167. Zech, H., and Vogt-Kohne, L., *Naturwissenschaften*, **11**, 337 (1955)
168. Loftfield, R. B., *Progr. in Biophys. and Biophys. Cytol.*, **8**, 347 (1957)
169. Chantrenne, H., *Ann. Rev. Biochem.*, **27**, 35 (1958)
170. T'so, P. O. P., and Squires, R., *Federation Proc.*, **18**, 341 (1959)
171. Gierer, A., *Z. Naturforsch.*, **13b**, 788 (1958)
172. Zaitlin, M., and Boardman, N. K., *Virology*, **6**, 743 (1958)
173. Uppal, B. N., *Indian J. Agr. Research*, **4**, 865 (1934)
174. Rappaport, I., and Wildman, S. G., *Virology*, **4**, 265 (1957)
175. Bennett, C. W., *Botan. Rev.*, **6**, 427 (1940)
176. Rappaport, I., Siegel, A., and Wildman, S. G., in *Physiological Triggers* (Am. Physiol. Soc., Washington, D.C., 1956)
177. Cohen, M., Siegel, A., Zaitlin, M., Hudson, W. R., and Wildman, S. G., *Phytopathology*, **47**, 694 (1957)

PECTIC AND CELLULOLYTIC ENZYMES IN PLANT DISEASE[1]

By R. K. S. Wood

Imperial College, London, S.W. 7., England

In most, if not all, plant diseases the pathogen penetrates or degrades the cell walls of host tissues at some stage. How this is done is one of the most important problems in pathogenesis. Although in some cases penetration may be wholly mechanical, in others it may be associated with some degradation of the wall. This degradation, and that which follows later will be caused by the action of enzymes secreted by the pathogen. Cell walls, even of undifferentiated tissue, have a complex structure and contain a wide variety of substances. Of the many enzymes which act upon the cell walls, only those with pectic substances and cellulose as substrates have been studied to any great extent and it is the significance of these enzymes in plant disease which will be considered in this paper. The degradation of textiles and wood will not be considered.

Before the enzymes are discussed, the structure of the cell wall and the nature and distribution of pectic substances and cellulose will be briefly described.

Cell Walls of Higher Plants

This subject has been reviewed comprehensively a number of times in recent years (1 to 5) so only a very brief account will be necessary in this paper.

The two parts of a dividing cell are separated in late telophase by the cell-plate which becomes the middle lamella when division is complete. The middle lamella may therefore be regarded as a layer common to adjacent cells; in young cells it is not known whether it is much different from the cell-plate. As the cell develops and increases in size a primary wall is laid down on the middle lamella and later, when the cells have stopped expanding, a secondary wall is deposited on the primary wall. In many mature, fully differentiated cells the secondary wall forms the bulk of the whole cell wall and has a laminated and complex structure.

The primary and secondary cell walls contain microfibrils embedded in a matrix continuous with the middle lamella. The microfibrils are of indeterminate length and from 50 to 250 A in diameter depending on their origin and treatment before examination. Most of them are believed to consist of cellulose but recently it has been suggested that some may consist of other polymers (6); it has also been claimed that the pectic substances of collenchyma have a microfibrillar structure (7) although this has been disputed by other workers (8). The arrangement of the micro-

[1] The survey of literature pertaining to this review was concluded June 1959.

fibrils in both primary and secondary walls is now known to be complex and to depend upon the plant species and type of cell (9 to 13). Much, if not all of the cellulose is believed to be in the microfibrils and these form a large proportion of the primary wall and still more of the secondary wall.

The middle lamella of young cells consists largely, if not entirely, of pectic substances. In secondarily thickened cells it becomes heavily impregnated with lignin and possibly other materials as well. The matrix surrounding the microfibrils of the primary wall also contains some pectic substances but it is now believed to consist mostly of hemicelluloses (14, 15). These are also found in the secondary wall but are relatively less important. The primary wall of young cells may also contain significant amounts of peptidic material (16), and recently it has been reported that there may be some protein in the middle lamella (17).

The matrix of the primary and secondary walls of older cells generally becomes impregnated with various substances. Lignin is by far the most important of these and is thought to be deposited around the microfibrils. The concentration of lignin decreases from the middle lamella to the lumen of secondarily thickened cells.

The general picture, therefore, is of a middle lamella composed mainly if not entirely of pectic substances, continuous with a matrix containing microfibrils, mostly of cellulose, but possibly of other polymers as well. The microfibrils become more abundant away from the middle lamella. In the matrix of both old and young cells, hemicelluloses probably predominate, but in young cells, pectic substances and proteins may also be important. In older cells lignin and other substances may be deposited in relatively large quantities and profoundly alter the properties of the wall.

In the rest of this paper only the pectic and cellulosic substances and the enzymes which act upon them will be dealt with in detail.

Pectic Substances in Higher Plants

This subject has been reviewed regularly for many years. Recent accounts are those of Kertesz (18); Lineweaver & Jansen (19); Hinton (20); Demain & Phaff (21); McCready & Owens (22); Henglein (23); and Deuel & Stutz (24).

The basic structure of pectic substances is a linear polymer of D-galacturonic acid units in α-1,4 glycosidic linkage. When the carboxyl groups on carbon 6 are free, the substances are known as polygalacturonic acids and when these are of high molecular weight they are known as pectic acids; pectic acid and the pectates formed with polyvalent cations are insoluble in water. Esterification of the carboxyl groups with methyl alcohol gives pectinic acids. The properties of pectinic acids of low methoxyl content approach those of pectic acid, but as the methoxyl content increases so does the solubility of the acids and of the pectinates formed with polyvalent cations. Pectinic acids of high methoxyl content, known as pectins, form firm gels with sugar and acid under suitable conditions. Pectins from

some sources, e.g., sugar beet, contain relatively large proportions of acetyl groups and generally do not form gels as readily as do other pectins. Pectinic acids are rapidly deesterified above pH 9.0.

Some of the pectic substances in the tissues of higher plants are extractable with cold water; these are generally high methoxyl pectinic acids or pectinates. Most pectic substances are insoluble in cold water, however, but can be extracted with solutions of acids and alkalis, and solutions of substances which form complexes with cations, particularly calcium and magnesium; extraction is more rapid and complete when hot solutions are used. The yield and composition of the pectic substances obtained from plants will depend upon the species, type of tissue, and methods of extraction; the yield is relatively much higher from young tissues. Preparations of pectic substances generally contain 20 per cent or more nonuronide material, most of which gives galactose and arabinose on hydrolysis. The proportion of uronide material can be considerably increased in various ways, but some arabinose and galactose generally remains in the final preparation unless this is so degraded as to have few of the properties associated with pectic substances. The consensus of opinion at the present time is that most of the galactose and arabinose is in the form of galactans and arabans which are mixed with the polygalacturonides or linked to them covalently. But there is also evidence from a number of sources for anomalous linkages in the chains and it is possible that these involve galactose and arabinose.

The precise location of the water-soluble pectic substances is not known but pectinic acids have been reported from cell vacuoles and it is possible that lower molecular weight pectinic acids occur in the cell wall itself, particularly in ripe fruit. The pectic substances of the middle lamella are insoluble in dilute acids but soluble in dilute alkalis. Based on this and other evidence they are believed to be complexes in which contiguous chains of pectic acid are linked through calcium, and to a lesser extent through magnesium and other polyvalent cations.

The rest of the insoluble pectic substances are known collectively as protopectin and most are in the matrix of the primary wall. They are soluble in dilute acids and yield pectinic acids with methoxyl content and properties dependent on the plant species and the type and age of tissue. It is not known why the native material is insoluble but possible reasons are: very high molecular weight of individual chains, cross-linkages between adjacent polygalacturonide chains through calcium or other cations, linkage between these chains and the occasional carboxyl groups which may occur in cellulose, hydrogen bonding, formation of anhydride bridges between chains, and admixture with hemicelluloses and other components of the matrix which may be poorly soluble in cold water.

Pectic Enzymes

Most of the reviews referred to in the last section also consider these enzymes. Two groups have been studied in detail and now are fairly well

characterized. The pectinesterases (syn. pectinmethylesterases) catalyze the hydrolysis of the methyl ester groups of pectinic acids to give methyl alcohol and pectinic acids of lower methoxyl content. Deesterification is rarely complete; even under optimal conditions some 10 per cent of the methyl ester groups are not hydrolysed. The enzyme is found in most parts of higher plants and is readily produced by many microorganisms. Enzymes from different sources have different properties, especially in relation to pH, activation by salts, and behaviour towards inhibitors. The action of pectinesterase is stated to be little dependent upon the molecular weight of the substrate but there are reports of interactions between the activity of this enzyme and that of the next group to be described.

The polygalacturonases break the polygalacturonide chains at the α-1,4 linkages to produce shorter chains and reducing groups. Demain & Phaff (21) distinguish between the polygalacturonases which have pectic acid or the deesterified parts of pectinic acids as substrates, and the polymethylgalacturonases which more readily attack the esterified parts of the chains. They further distinguish between endo-types of polygalacturonases and polymethylgalacturonases in which the glycosidic linkages are broken at random, and the exo-types in which the end linkages are preferentially attacked.

Deuel & Stutz (24) classify the polygalacturonases somewhat differently into Type I, the liquefying polygalacturonases, which breaks the glycosidic linkages at random and is more active on the lower methoxyl pectinic acids; Type II, α-pectin glycosidase, which again breaks the chain at random but which attacks high methoxyl pectinic acids more readily; and Type III, saccharifying polygalacturonases, which attacks end linkages only. There is not much difference between these two systems but the first will be used in this paper because it is somewhat more complete.

The action of some preparations on pectic or pectinic acids produces neither the monomer nor any oligomers and the term "depolymerase" has often been applied rather uncritically to enzymes which act in this way. Some of the described effects could follow the action of polygalacturonase-type enzymes on substrates which are not esterified in the right way and when this happens the term is superfluous and sometimes misleading. It is also unnecessary to use the term when, although the monomer is not produced, a large proportion of the glycosidic linkages are broken. If the term is used at all it is best confined to instances when the enzyme action is limited to a very restricted hydrolysis of the substrate, say 5 to 10 per cent. These enzymes will undoubtedly be reclassified when more is known about the nature of the linkages which are attacked.

Finally there are the hypothetical enzymes known as protopectinases, which logically have protopectin as substrate, i.e., the native pectic substance in cell walls which gives soluble pectinic acids on hydrolysis. Sometimes, however, the term is used for the enzyme or group of enzymes bringing any of the insoluble pectic substances of the cell wall into solution

and so would include the enzymes which degrade middle lamella pectates. The activity of this enzyme has also often been equated in the past with that of the ezymes which "macerate" plant tissue, the process in which the cells become separated and the tissue loses its coherence. Although maceration may include the action of protopectinase, whether this term is used in the more restricted or wider sense, it may also include many other activities so that it would be better not to use protopectinase in this connection until it is established that the essential feature of maceration is the degradation of protopectin.

Production of Pectic Enzymes by Plant Pathogens

Production in vitro.—Many plant pathogens readily produce pectic enzymes *in vitro* and there is extensive work on how cultural conditions affect enzyme secretion. Relatively little is known about the intracellular pectic enzymes although these were the ones used by some of the earlier workers in studies of maceration (25). The results obtained for pectinesterase are rather readily assessed because the effect of substrate is circumscribed and the mode of action is not greatly affected by other pectic enzymes. But with the chain-splitting enzymes the results are often difficult to interpret because in much of the reported work no attempt has been made to specify the type of activity which was measured, and no allowance has been made for the effects of different pectic enzymes upon one another. There are still greater difficulties when maceration is the activity studied because here other types of enzymes may also be involved. In spite of these uncertainties some general points have emerged.

Pectinesterase is produced by many fungi and bacteria and in some cases secretion either depends on or is greatly stimulated by the presence in the medium of the specific substrate (26 to 29). The results obtained with the nonpathogenic *Penicillium chrysogenum* are also interesting in this respect because pectic, D-galacturonic, L-galactonic, and mucic acids stimulated enzyme secretion although none of these substances contain the linkage attacked by the enzyme (30). Somewhat similar results were obtained with the pathogen *Pseudomonas prunicola* (31).

In other examples, pectinesterase is secreted abundantly in the absence of substances closely related to pectinic acids (32). This was used to obtain pectinesterase free from chain-splitting enzymes.

Some pathogens produce little or no pectinesterase in culture even when pectinic acids are present, although in the same cultures chain-splitting enzymes are produced abundantly (33 to 41). Too much importance need not be attached to negative results of this sort because different isolates of the same species may behave quite differently. Thus some workers (35, 42) have found that *Erwinia aroideae* does not produce the enzyme in culture, but another worker found that culture filtrates gave positive results for enzyme activity after incubation with the substrate for 24 hr. Although this might have meant that the activity was in fact very low (43), it would

be surprising if different isolates did not differ considerably in their ability to produce this and other enzymes.

Specific activities have been considered in few of the corresponding studies with polygalacturonases. Activity has been measured all too frequently only in terms of viscosity changes so that the data obtained do not show which type of enzyme has been studied. Moreover, the implications of the presence of pectinesterase in the culture filtrates has sometimes been ignored. In spite of these limitations some conclusions can be reached.

Some pathogens produce chain-splitting enzymes in response to pectic substances (26, 27, 28, 36, 40) and to substances such as galacturonic acid (40), whereas others produce active filtrates even when this type of substance is absent (42, 44). Sometimes pectic substances in the media reduce secretion of enzymes but this can be regarded as exceptional. Most pathogens which produce these enzymes in culture do so readily under a variety of conditions but there are some with rather exacting requirements (45, 46).

There is little evidence that any of the products of hydrolysis of pectic substances has any great inhibitory effect on enzyme secretion; on the contrary, it has been shown somewhat unexpectedly that the ultimate product of enzyme activity, galacturonic acid, may stimulate enzyme secretion (30, 40).

As might be expected, secretion of polygalacturonases is often little related to general growth of the pathogen and it is possible to select cultural conditions in which growth is poor but enzyme secretion is high. Enzyme production may also be affected by the C/N ratio of culture media; this is not surprising because it will involve the secretion of relatively large quantities of protein.

The effects of cultural conditions on the secretion of macerating enzymes has often been studied (40; 44 to 50) but the results are generally difficult to interpret because of the difficulties mentioned above. However, it has now been well established for some organisms that the macerating activity of culture filtrates obtained in various ways follows the activity of the chain-splitting enzymes, and that the properties of the enzymes responsible for the two activities are similar (42, 45). But with other organisms this is not so, maximum activity of the macerating enzymes and of pectinesterase and polygalacturonases, being obtained under different conditions (51).

A possibility revealed by *in vitro* studies is that the properties of some of the enzymes may be modified by the conditions under which they are secreted (46, 52, 53). The evidence so far obtained is little more than suggestive but this aspect needs to be investigated further because of its significance in the development of disease.

Little is known about the types of cells in fungal and bacterial cultures that most actively secrete pectic enzymes, although it is often assumed that the young, actively growing cells are most important in this respect. But this may not always be so because the enzyme activity of cultures sometimes

continues to increase after active growth has stopped and enzyme secretion is often good when conditions do not permit rapid growth of the pathogen. The course of enzyme secretion needs to be studied further because in the initial stages of infection the rapidity with which enzymes are produced may be important in determining whether or not a progressive lesion is produced. Bacteria are particularly suitable for this type of investigation and species which rot potato tubers have been used in some preliminary studies showing that significant quantities of enzyme are secreted before active bacterial growth begins (53). This may also happen with fungal spores and very young germ tubes and play an important part in the earliest stages of infection.

In the light of what is now known about pectic enzymes and the factors affecting their production, the following points should be taken into account in future studies. With polygalacturonases the type of activity being assayed must be specified and, when possible, substrates must be used which are not affected by other enzymes that might be present; otherwise it is necessary to get rid of contaminating enzymes and this is generally difficult and laborious. In the production of enzymes, the type of substrate and the conditions within the culture must be carefully considered. For example, when pectinic acids are added to media they send the pH to low values and if pectinesterase is secreted, acid is produced which may reduce the pH to values unfavourable for growth of the pathogen. Age of the culture and adsorption of the enzymes by the pathogen must also be reckoned with in addition to possible effects of the cultural conditions on properties of the enzyme, particularly the effect of pH on activity.

Surveys of large groups of pathogens for their ability to secrete pectic enzymes are generally of limited value because different isolates of the same species may behave quite differently. The limited range of cultural conditions practicable in most surveys also lowers the value of much of this work. Detailed studies with single isolates of known pathogenicity are likely to be more rewarding especially if these are associated with and used to clarify investigations of the activity of the pathogen *in vivo*.

Production in vivo.—Dimond & Waggoner (54) have given the conditions that must be satisfied before specific toxins known to be produced by a pathogen *in vitro* can be assumed responsible for symptoms of disease produced by the pathogen in a particular host. Similar arguments apply to pectic enzymes. The demonstration that a pathogen produces one or a variety of pectic enzymes in culture may not mean very much, particularly when highly degraded substrates are used to measure enzyme activity. From a plant pathology point of view it is somewhat more significant if the culture filtrates are also able to macerate plant tissue, but even this is not always conclusive. This is illustrated by recent work with bacteria attacking potato tubers in which certain nonpathogenic bacteria produced macerating enzymes more readily and abundantly on a variety of extracts of tubers than did a pathogenic species (53, 94).

The only satisfactory proof that pectic enzymes play a part in the development of a disease is by demonstrating that they are, or have been, present and active in the plant tissue. Evidence for activity can sometimes be obtained microscopically because in some diseases the cells of invaded tissue separate along the line of the middle lamella and this layer and other parts of the cell wall lose their ability to be stained with reagents which colour pectic substances. Unfortunately most of the reagents used in the past, particularly ruthenium red, are by no means specific for pectic substances and results based on their use must be interpreted with caution. Another more specific reagent has recently been described (55, 56); it has already been used in anatomical studies with interesting results (57), and may prove valuable in similar studies with diseased tissue.

A better, but still indirect approach is to compare quantities and types of pectic substances in both rotted and adjacent uninvaded tissue. Disappearance, or changes in the nature of pectic substances is strong although not conclusive evidence that pectic enzymes of the pathogen have been active. Tissue of apples rotted by different fungi have recently been studied this way (48, 58).

The best way to demonstrate the importance of pectic enzymes in a particular disease is to show that diseased tissue contains significant quantities of these enzymes with properties different from those present in healthy tissue. It should also be shown that the presence of the enzymes can be correlated with known changes in the composition and concentration of the pectic substances (58). Only in a very few plant diseases has this been attempted in spite of the fact that in one group, the soft-rots, pectic enzymes can easily be obtained in relatively large quantities from rotted tissue (41, 58, 59). But even with favourable conditions the results obtained may be difficult to interpret. Thus, many types of healthy plant tissue contain large quantities of pectinesterase so that the activity of the indigenous enzyme in rotted tissue must be separated from that of any pectinesterase produced by the pathogen. This would not always be easy, but in one case it has been done by making use of the different susceptibilities of the two types of enzyme to inactivating agents (32). The fact that pectinesterases from higher plants and from microorganisms often differ in their reaction to pH might also be used for the same purpose.

In some work the fact that a large proportion of pectinesterase is generally strongly adsorbed by insoluble parts of host tissue has been overlooked. Enzyme adsorbed in this way is released in salt solutions at pH 8.0 to 9.0. At present it is not possible to assess the relative importance of free and adsorbed enzyme, but it is probably safer to assume that adsorbed enzyme is active and to express results in terms of the total enzyme present. In this connection, it should also be remembered that enzymes are probably more easily extracted from rotted tissue in which most of the cells are separated from each other than from healthy tissue unless this is disintegrated to a similar degree.

Indigenous enzymes are far less troublesome when the chain-splitting

enzymes are considered, because although they have been reported from a variety of plants, activity is usually low compared with that produced by pathogens. With these enzymes the main difficulties arise from the fact that the wide variety of substrates present in plant tissue may lead to the production of different types of polygalacturonase (58), and of other enzymes which may affect the activity of the polygalacturonases when the usual substrates are used for assay because these almost always contain contaminating substances. This may account for the fact that some organisms produce macerating enzymes much more readily on natural substrates than in liquid culture (46), and it has been shown that *Penicillium expansum* produces different types of polygalacturonase in apples, and in culture media with apple pectin as the carbon source (58).

Failure to obtain active extracts from rotted tissue even after using a variety of methods does not necessarily mean that pectic enzymes played no part in pathogenesis. They may have been produced and been active with significant results, but later may have been inactivated by substances present in healthy plants or produced in rotted tissue after the cells have been killed (58). A number of naturally occurring inhibitors of pectic enzymes are now known and will be dealt with later.

When diseased plants are examined for pectic enzymes it is also important that the tissue should be at the right stage of development of the disease. This is a factor because pectic enzymes are probably most important in the earlier stages of tissue breakdown and because it is the young, actively growing cells of pathogens which probably produce enzyme most freely. It would be better, therefore, to look for pectic enzymes in the most recently invaded tissues although it may then be difficult to get sufficient quantities for characterization. In the later stages of disease development it may be easier to find and isolate pectic enzymes but then they may be less significant as shown by certain wilt diseases in which tissue breakdown is a late and probably not very important symptom. Also, in the later stages contaminating organisms may complicate the picture, particularly in tissues such as those of potato tubers which are readily attacked by a variety of saprophytic bacteria after the initial barriers to infection have been broken down by primary pathogens. These bacteria may produce large quantities of pectic enzymes within a few hours of beginning active growth. Such difficulty will be important when the significance of pectic enzymes in diseases of roots is investigated. Another disadvantage of using tissue which has been colonized for some time is that inactivation will be more important when enzyme inhibitors are present.

Action of Pectic Enzymes on Plant Tissue

This section will deal primarily with the action of pectic enzymes on parenchymatous cells and other cells with poorly developed secondary walls and in which the cell wall as a whole has not yet become heavily impregnated with substances such as lignin and suberin.

In the earliest stages of infection a broad distinction may be drawn be-

tween pathogens which only invade plants through wounds and those which are able to become established in undamaged tissue; later the two types of pathogens often behave similarly. It may be that the differences in the early behaviour of the two types depends on the ability of one but not the other to mechanically penetrate outer barriers such as the cuticle (60). The possibility also exists, however, that pectic enzymes are important in the earliest stages of infection and that the wound parasite produces these enzymes only when pectic substances are present, whereas the other type of pathogen behaves otherwise. That this may be so is suggested by work done some time ago on the infection of citrus fruit by the wound parasite *Penicillium italicum* which readily invaded undamaged fruit when pectic substances, but not other materials, were added to the inoculum (61). The water-soluble pectic substances present in most plant tissues may also be important in stimulating enzyme secretion even by pathogens that do not depend on wounds. The process of secretion, once started, becomes cumulative because the enzyme activity releases substances which permit further growth of the pathogen and still further stimulate enzyme secretion.

Nothing is known about the part played by indigenous enzymes, particularly pectinesterase, in the early stages of infection nor about the importance of enzyme adsorption by host tissue. In this connection the possibility that the pathogen produces substances which affect the degree of adsorption should not be overlooked because recent work has shown that substances such as indoleacetic acid, known to be produced by some pathogens, affect the binding of pectinesterase to the cell wall (62 to 66).

In the earliest stages of the attack of parenchyma the cells separate along the line of the middle lamella and this is soon followed by death of the cells. The separation may not be very obvious if the tissue is left *in situ* but in water the tissue soon loses most of its tensile strength and the cells separate readily. At this stage the cells retain their identity and it would seem that suspensions obtained in this way might be used in some types of studies of cell metabolism. If it is assumed that the middle lamella consists essentially of pectates, these would be degraded by the different types of polygalacturonase although the process would be impeded by the insolubility and inaccessibility of the substrate. Presumably, as linkages within the chain are broken, those between the chains become less effective until the coherence of the gel is lost, but this needs to be investigated further in view of the insolubility of some of the double salts of galacturonic acid. Substances that sequester calcium and magnesium have been used for maceration but they act much less rapidly than do active preparations of macerating enzymes; it would be interesting to know how these two types of substances would act together.

The middle lamella is continuous with the matrix of the primary wall and it is difficult to imagine an abrupt change in the nature of the pectic substances between the two regions, so it is possible that maceration could follow the degradation of pectic substances in the outermost layers of the

matrix. That this is true is suggested by results obtained with filtrates from cultures of *Pythium debaryanum,* a fungus causing a soft-rot of potato tubers and many other types of tissues (59). These filtrates do not attack pectates *in vitro* although they macerate tissue readily and degrade pectinates of high methoxyl content to relatively high molecular weight substances. Alternatively, it must be assumed that the middle lamella contains high methoxyl pectinates, whose breakdown leads to maceration.

After the cells have separated, enzymes produced by the pathogen continue to act upon the pectic materials of the primary wall bringing the protopectin into solution. As already stated, it is not known why protopectin is insoluble but part of the insolubility may depend upon regular but infrequent cross-linking between the free carboxyl groups of adjacent chains of pectinic acids. If so, then the mode of degradation could be similar to that of the middle lamella because linkages within the chain would be broken by polymethylgalacturonases, or by polygalacturonases after hydrolysis of the esterified parts of the chains by pectinesterase already present in the tissue or produced by the pathogen. Based on these suppositions there is no reason why the pectic materials of the primary wall and of the middle lamella, should not be degraded by the types of enzymes already well characterized. This view has been taken by a number of workers using indirect evidence. More directly it has been shown that cuticle may be separated from the rest of a leaf with a preparation of endopolygalacturonase stated to be 95 per cent pure obtained from a yeast (67). There are other examples along similar lines (68, 69). It has also been suggested, however, that other types of enzymes may be responsible for the earliest stages of degradation of native pectic materials, assuming that linkages exist in pectic and pectinic acid chains besides those between the primary units. Such linkages are still to be demonstrated. But if they do occur, and are spaced infrequently though regularly it is possible that there are also enzymes which attack them to release relatively high molecular weight substances which then would be degraded by polygalacturonases. Until recently there was only indirect evidence for enzymes of this sort but lately it has been reported that an enzyme has been isolated from cultures of *Clostridium felsineum* by ion-exchange chromatography which produced soluble pectin from insoluble forms but did not attack pectates or pectin in solution (70). Somewhat similar results have been described for enzymes secreted by *Aspergillus kawachii* (71).

There is now another possibility to consider, namely, that most of the pectic substances of the cell wall are insoluble because they are linked in some way with, or surrounded by, other substances which are insoluble. Solution would then depend on the breaking of these linkages or degrading the other substances to soluble compounds. This could happen even in the middle lamella which until recently has been considered essentially homogeneous. It has now been shown to contain a small proportion of protein (17) and this, clearly, could profoundly alter the susceptibility of the

middle lamella to attack by pectic enzymes, because the pectates would be protected until the protein had been removed by the action of proteolytic enzymes. These are produced freely by many microorganisms but they might be removed when polygalacturonases are purified so that a consequent loss of an ability to macerate could be related to this and not to the elimination of other pectic enzymes.

When the matrix of the primary wall is considered, a variety of substances other than pectinic acids are involved. Maceration, if it involves this layer, and subsequent degradation of the wall, might depend to some extent upon enzymes able to degrade arabans, galactans, hemicelluloses, and proteins, particularly if these are evenly dispersed. Degradation of these substances would, in turn, be affected by the action of pectic enzymes. In view of the importance of substances other than those of a pectic nature in the young cell wall, it is surprising that the enzymes which degrade them have been so little studied. These considerations apply even more when the middle lamella and the matrix of the primary and secondary walls become impregnated with substances such as lignin and suberin.

Finally, pectic substances may not be of the same nature in different plant species, in different parts of a given plant, and in the same parts of a plant at different stages of development. These differences will undoubtedly affect the susceptibility of the tissues to degradation.

Killing of Plant Cells by Pectic Enzymes

Some of the earliest and best work on this subject was reported by Brown in 1915 (*25*), and not much more has been learned since. During maceration it is known that cells are rapidly killed and with the exceptions given below the principles causing maceration have not been separated from those which kill the cell (*72*, *74*). At present, therefore, it can be assumed that they are the same, or with very similar properties, or that the hypothetical toxin cannot act until the cells have been acted upon by the macerating enzymes. Assuming that there are no separate toxins in the limited sense, then irrespective of the types of enzymes involved it is rather remarkable that the limited degradation of the cell wall in the early stages of maceration should kill the cell because it is unlikely that the products of hydrolysis are toxic in the concentrations produced (*72*) and because it is not difficult to obtain from untreated cells free protoplasts which remain alive for some time (*74*). Moreover, recent work has indicated that the outer layers of the protoplast are sharply separated from the wall of the cell except at the plasmodesmata (*11*) making it difficult to see why dissolution of the middle lamella should much affect the relation between the protoplast and the wall if the tissue is undisturbed during maceration. It is possible that macerating solutions contain proteolytic and other enzymes which might damage the protoplast after the middle lamella had been degraded and in this connection the report that gelatin reduced the toxicity of some enzyme preparations may be significant (*75*). But macerating solutions

that killed plant cells and had no proteolytic activity have also been described (73).

There have been two reports of the separation of macerating activity and toxicity (44, 74). The first of these is the more important and showed that when tissue slices are put in solutions containing macerating enzymes and substances such as sucrose or potassium nitrate in concentrations just sufficient to cause plasmolysis, the rate of maceration is only slightly reduced, but killing of the cells is greatly retarded. This effect has been obtained with different tissues and with preparations from different organisms; as yet it is unexplained (72, 74).

Inhibitors of pectic enzymes.—As a group, the pectic enzymes are rather resistant to many substances which inhibit the action of other enzymes (76, 77). In a recent survey it was found that none of a wide range of fungicides was particularly inhibitory to the polygalacturonase of *Cladosporium cucumerinum* (38). But surface-active agents inactivate pectinesterase from higher plants (76), and polygalacturonase is known to be inhibited by formaldehyde and tannins (78). The action of these substances is probably not very specific. More recently, inhibitors with more specific effects have been reported from a number of sources (48, 58; 79 to 91). Most of these are polyphenolic substances or oxidation derivatives. It has been shown that some of these substances affect some pectic enzymes but not others, and that they probably play an important part in the pathogenesis of certain rots of apples (48, 58, 81, 82, 91). There is good evidence that the oxidation of phenols in certain varieties of apples produces high molecular weight, tannin-like substances which inactivate the enzymes responsible for maceration and so limit or prevent infection (81, 82). It has also been demonstrated that the products formed by oxidation of the leuco-anthocyanins of apples in some types of rots prevent the degradation of pectic substances which occurs in other rots where, for unknown reasons, this oxidation is prevented (58, 91).

Inhibitors of pectic enzymes are probably widespread in plant tissues and important in resistance and it is possibly significant that there is growing evidence of resistance often being linked with phenolic substances in plant tissues.

Pectic Enzymes in Different Plant Diseases

Soft-rots and related diseases.—The pectic enzymes of plant pathogens are best known in relation to diseases in which a relatively large mass of parenchyma is rapidly invaded to give a mass of water-soaked tissue generally soft in texture and with little coherence. The changes in colour associated with oxidative processes in tissue killed in other ways often do not take place, the tissue remaining colourless as in rots of apple caused by *Penicillium expansum* (58) and of potatoes caused by *Erwinia aroideae* (42).

Some soft-rot organisms produce pectinesterase in culture (26, 51, 83),

but others do not, at least not readily (34, 35, 42). This is not to say that the enzyme is not involved in pathogenesis because most tissues of higher plants contain large amounts of pectinesterase. It is also possible that a pathogen may produce the enzyme *in vivo* although not *in vitro*. Not much is known about its role in pathogenesis but because it is so widely distributed in host tissue the production of it by pathogens may not be as important as is sometimes assumed. Any consideration of it in relation to disease will have to take into account the effects of other substances on its adsorption by cell material (62 to 66).

A number of polygalacturonases have been described in connection with a variety of soft-rot diseases (26, 34, 35, 40, 41, 51, 83, 92). Many are still poorly characterized and inadequately studied; however, it is clear that apart from differences in mode of action, which will be limited in number, enzymes from different sources vary considerably in physical and chemical properties. It has already been mentioned that some of these properties may depend on the conditions under which the enzymes are produced (46, 52, 53). Only detailed and comparative study will reveal which of these differences are important in pathogenesis.

Parenchymatous tissue differs greatly in susceptibility to attack by soft-rot organisms. Some of the factors involved are as follows: (*a*) increasing the water content of tissue greatly increases its susceptibility to attack by soft-rot pathogens and also augments the rotting caused by macerating enzymes (50, 93, 94, 95); (*b*) the pectic substances in different tissues may differ considerably in composition and this may affect susceptibility to degradation, e.g., pectinic acids of beet contain a large proportion of acetyl groups (96) and the pectinic acids of young peaches are almost completely esterified (57); (*c*) the structure of the pectic substances and, therefore, the consistency of the cell wall, may be affected by nutrition, particularly by the availability of calcium and boron during growth (97 to 104), and by growth-regulating substances (103; 105 to 108), and this may alter their susceptibility to attack by pectic enzymes; (*d*) some types of parenchyma may contain inhibitors of pectic enzymes that act relatively quickly and so prevent infection, or act more slowly and so retard the degradation of the pectic substances in the cell wall.

Although the importance of pectic enzymes in soft-rot diseases is now well established, much remains to be learned about their precise role and their activity in relation to that of enzymes acting upon other components of the cell wall. Mutants with different abilities to produce pectic and other enzymes, and with various pathogenicities, would be useful for comparative studies and have already been used in one investigation (109). The significance of the ability to secrete pectic enzymes must not be overestimated as a mechanism in pathogenesis, however, because many other factors will be involved and only some of these will act by affecting the secretion or activity of these enzymes (53, 94, 95).

Leaf-spot diseases.—In this and related types of disease the pathogen

spreads only to a limited extent in parenchymatous tissue and produces a small area of dead cells. The effects are similar to those often seen when soft-rot pathogens attack resistant tissue, and it may be significant that some leaf-spots begin to have the characters of soft-rots when conditions are particularly suitable, e.g., when the host tissue is water-soaked. The type of lesion produced is generally attributed to the production by the pathogen of a toxin but this does not explain why the pathogen normally develops to such a limited extent. Although the subject has been little studied, it is probable that many leaf-spot pathogens are able to secrete pectic enzymes so that it would be interesting to discover if the enzymes are produced in diseased tissue and if so, why they have such slight effects on the host under normal conditions.

Vascular wilts.—Although the physiology of these diseases has been studied for many years, the possibility that pectic enzymes are involved in the development of disease symptoms has been considered only relatively recently.

Three stages in the development of the disease can be distinguished. The first stage, that of entry into the vascular elements, has been little studied but because it may involve penetration of the tissues outside the vascular elements, cell wall degrading enzymes may be important in it. During the second stage the pathogen grows in, and is confined to, the vascular elements. Most of the typical disease symptoms are associated with this stage and it is also the most studied. In the final stage, when the plant is moribund, the pathogen may move out of the vascular elements and cause some breakdown of the adjacent tissue, a feature absent from the earlier stages (110).

Evidence that pectic enzymes play a part in the disease development comes from several sources and may be summarized as follows. (*a*) The pathogens often readily produce pectinesterase, polygalacturonase, and macerating enzymes in culture, generally but not always, in response to specific substrates (32, 33, 36, 37, 39, 44, 110, 111, 112). (*b*) Preparations with pectic enzyme activity can produce in cuttings some of the natural disease symptoms; pectinesterase activity has been considered important in this connection (112 to 114). (*c*) In some diseased plants there is evidence for the action of pectic enzymes particularly in the final stages of the disease (110, 113, 115). (*d*) Pectinesterase believed to have been produced by the pathogen has been obtained from the vascular sap of diseased plants (32) and extracts from the tissues of diseased plants have more pectinesterase activity than those from healthy plants (29).

Other facts to be considered are these. (*a*) Chain-splitting or macerating enzymes have not so far been obtained from plants in the second stage of disease. These enzymes can be detected in very low concentrations so that if they are produced it should be possible to demonstrate them or to show that they can be inactivated by host tissue after being produced. In this connection it would probably be more useful to examine vascular tissue *per se* for these enzymes as well as the exudates from cut shoots because

presumably these exudates will come from elements little affected by the pathogen and because some of the enzyme may be adsorbed by the walls. (*b*) Although vascular elements may be blocked by large molecular weight pectic substances released from the walls by pectic enzymes (32), it must be remembered that if the action of the enzymes is continuous these substances would be degraded to low molecular weight compounds which might be innocuous. (*c*) In some wilt diseases preparations with pectic enzyme activity do not produce typical disease symptoms (110, 111), although they produce other symptoms which showed that the enzymes had moved through the vascular system (110). (*d*) Some of the known physiological effects of the pathogen on the host are not reproduced when cuttings are treated with pectic enzymes (116). (*e*) Pathogenicity may be little related to the ability of different isolates to produce pectic enzymes *in vitro* (110, 117).

In summary, therefore, it may be said that whereas good evidence exists that pectic enzymes are important in the final stages of some diseases, so far there is no conclusive evidence that they are important in the penetration phases, nor in the second stage which is the one associated with the most characteristic symptoms.

It must be remembered, however, that the problem has received relatively little study so far as pectic enzymes are concerned and certain aspects have been almost ignored. For example, in many wilt diseases there is only a limited development of the pathogen in the plant so that only very small quantities of extracellular substances will be produced. But if these are enzymes, and if they are not inactivated, very small quantities would be sufficient to produce quite large effects over the relatively long time required for typical wilt symptoms to appear. And, of course, it is possible that the important effects are those produced at a distance from the site of enzyme secretion, possibly in the leaves themselves, particularly because substances exposed at the internal surfaces in xylem elements, except possibly at pits, would not seem particularly suitable for the activity of pectic enzymes. There is indirect evidence for the action of pectic enzymes at a distance in the silver leaf disease caused by the fungus *Stereum purpureum* (118).

Other diseases.—Many obligate parasites, and some nonobligate types too, grow between the cells of invaded tissue. This type of parasitism is not normally associated with any marked degradation of cell walls, but some recent work has raised the possibility that even in this sort of disease cell wall degrading enzymes may play a part. It has been shown that germinating uredospores of *Puccinia graminis tritici* produce a variety of enzymes that act upon different components of the cell wall and, in particular, that pectic enzymes were produced when pectin was present (119).

Although the infection of roots by nodule bacteria is not considered a disease in the usual sense, it may be mentioned in passing that there is now

evidence that pectic enzymes may play a part in the establishment of these bacteria in plants (120).

Cellulases

These will be treated much more briefly because most of what we know about them comes from studies with organisms that degrade textiles and wood and are not plant pathogens in the more restricted sense. It will be necessary to refer to some of this work because comparatively little is known about the cellulases of plant pathogens. Recent reviews dealing with these enzymes are those of Siu (121); Siu & Reese (122); Greathouse & Wessel (123); Jermyn (124); Reese (125); and Cowling (126).

Forms of Cellulose

The basic unit of cellulose is a linear polymer of glucose residues linked by 1,4-β-glycosidic bonds. Aggregates of 50 to 100 chains are produced by hydrogen bonding or by the action of van der Waals' forces between the chains. In the crystalline parts of these aggregates the chains are highly orientated, in the amorphous parts they are less well ordered and the two regions are not necessarily well separated. The aggregates are organised into microfibrils of indeterminate length and 50 to 250 A in diameter. The microfibrils are readily seen by electron microscopy; between the fibrils there are spaces some 100 A in diameter which may be continuous with intrafibrillar spaces some 10 A in diameter. Within both types of spaces there may be substances other than cellulose. It is possible that there are anomalous if infrequent linkages within the individual chains and occasional linkages between these chains and other substances. Recent work has also shown that polymers other than those of glucose may form microfibrils (6).

In the forms described above, cellulose is insoluble but may be made soluble by replacing the hydrogen of the primary and secondary hydroxyl groups with methyl, ethyl, carboxymethyl, or other groups. These soluble derivatives have been much used in enzyme studies. Native cellulose may also be modified in other ways that greatly alter its susceptibility to enzyme action, e.g., by mechanical degradation or irradiation.

Enzymes attacking celluloses.—Only those of a few fungi have been studied in detail and in spite of intensive studies, sometimes with the same species, there is still no general agreement as to how native cellulose is degraded. The different views may be summarized as follows. (*a*) Only one enzyme degrades insoluble cellulose to glucose (127 to 129). (*b*) One enzyme degrades insoluble cellulose to cellobiose and another produces glucose from the disaccharide (130 to 132). (*c*) A number of enzymes are involved (125; 133 to 138). One called C_1 releases individual chains from native cellulose; the chains are then hydrolysed by a series of enzymes known collectively as C_x to cellobiose or glucose. Cellobiose is finally de-

graded to glucose by cellobiase or other β-glucosidases (125, 136, 137, 138). The mechanism of the action of the C_1 enzyme is obscure. It may be a C_x enzyme acting specifically on particularly long chains or it may act upon occasional linkages or minor components in native cellulose. It could also be identical with the swelling or S factor (139, 140) which causes cotton fibres to swell in dilute alkali.

Production of Cellulases

From the point of view of plant pathology, the most important facts which emerge from studies mainly concerned with organisms that are not plant pathogens are given below.

Almost always in the absence of soluble or insoluble celluloses no, or very little, enzyme is secreted (121). In this respect cellulases as a class differ from the pectic enzymes whose production is often constitutive. The problems connected with the induction of enzyme secretion by insoluble cellulose have been little studied to date. It is possible that even when secretion is highly adaptive some enzyme is secreted and that this releases fragments sufficiently small to pass into the cells of the organism where they would induce the formation of more enzyme, the process then becoming rapidly cumulative. On the other hand, it has been suggested that the first stages in cellulose breakdown involve enzymes having particularly large molecules as specific substrates. It is not easy to see how these could get into cells, and, therefore, how they induce enzyme formation which presumably must take place within the cell membrane. Much more work is needed on the induction of enzymes by insoluble substances because the degradation of insoluble substances is important at many stages of infection.

Under some conditions the products of cellulose hydrolysis reduce or prevent enzyme secretion (141). This is another point of difference with the pectic enzymes, and clearly could be significant in pathogenesis because cellulases would not be secreted in the presence of other readily available carbon sources. It may also account for the fact that when such sources are absent or limited, and consequently growth is poor, enzyme secretion is often high.

Misleading conclusions might be drawn from results obtained with cell-free cultures filtrates because even under optimal conditions these degrade celluloses much more slowly than do growing cultures under the same conditions (125). Reasons for these differences are not known but it is clear that degradation of cellulose *in vivo* may proceed much more rapidly than would be expected from the activity shown by culture filtrates.

Although hundreds of organisms, many of them plant pathogens (33, 38, 41, 110, 111 and 142 to 149), are now known to produce enzymes which degrade soluble celluloses, only a relatively small proportion are able to deal with insoluble cellulose even after it has been physically or chemically modified to make it more susceptible to enzyme action (33, 38, 145, 146, 148, 149). In the study of pathogenesis, it is the breakdown of insoluble cellulose

that is important, therefore, investigations confined to breakdown of soluble derivatives have only a limited value. But the convenience of these substrates makes them useful for preliminary experiments because if an organism does not produce enzymes that degrade these substances, it is unlikely to produce enzymes which act upon insoluble celluloses.

Very little is known about the production of cellulases by plant pathogens *in vivo*, and only isolated attempts have been made to extract them from diseased tissue (145). It has been shown, however, that either they are not present or they are not easily extracted from some types of rot (58). But in some diseases cellulases have been obtained from invaded tissues (145) and microscopical evidence for cellulose degradation in the later stages of disease has also been obtained (110).

Significance of Cellulases in Different Plant Diseases

In soft-rots the most important stages are those in which the middle lamella and adjacent layers are degraded so that the cells separate from one another, although the cell wall remains essentially intact. It is known that culture filtrates which macerate plant tissue need not have any cellulase activity (58) and, conversely, filtrates may have a high cellulase but a low or no pectic enzyme activity (136). It is unlikely, therefore, that cellulases play any part in the early stages of maceration. This is to be expected from what is known about the structure of the cell wall and the conditions which govern the production of cellulases by microorganisms. Secretion is almost always induced by the substrate. This is not present in the layers degraded in maceration and would probably not be exposed until the matrix around the microfibrils had been removed by the action of other enzymes produced by the pathogen. There is also the point that even when the substrate is available, secretion of the enzyme may be retarded or prevented in the presence of other, readily available, carbon sources such as would come from the cell vacuole and hydrolysis of the matrix substances. But it must also be remembered that the cell walls *in situ* will be highly hydrated and that in this condition the matrix may not be as significant a barrier to the movement of enzymes.

At later stages in soft-rot diseases the cellulose of the microfibrils is undoubtedly attacked because some soft-rot pathogens are actively cellulolytic. The nature and course of the attack has been little studied, however, and practically nothing is known about the production of cellulases *in vivo*. Under natural conditions it is likely that much of the cellulose is degraded by secondary invaders which are weak parasites but strongly cellulolytic, e.g., *Trichoderma viride*.

Cellulases may be more significant in diseases where the pathogen moves only relatively slowly through the host tissue. In these diseases there is a slow but more complete degradation of the tissue by the primary pathogen so that the lesions are quite different in form from those found in soft-rots, e.g., the dry-rot lesions in potato tubers caused by *Fusarium caeruleum*

compared with the watery rot caused by *Pythium debaryanum*. But these diseases have been little studied from this point of view and would not be investigated easily because of the slowness with which lesions develop and the difficulties of obtaining reasonable quantities of enzyme from diseased tissue.

There is now some evidence that cellulases may play a part in the development of some wilt diseases (110, 111, 146). Species of *Verticillium* and *Fusarium* which cause wilt diseases produce cellulases freely in culture and are probably truly cellulolytic (146, 149). *Pseudomonas solanacearum*, the causal agent of a wilt disease of tobacco, produces a C_x type enzyme in culture, and evidence was obtained for degradation of cellulose *in vivo* (110). Also, it has been shown that a solution of a purified cellulase from *Myrothecium verrucaria* caused tobacco plants to wilt before, but not after, the enzyme had been inactivated by heat (110), and a similar result was obtained with a partially purified preparation from cultures of *Fusarium oxysporum lycopersici* (146).

With the limited data now available, it is difficult to assess the significance of cellulases in the vascular wilts. Although there is no direct evidence that they are produced *in vivo*, conditions in the tracheal elements would not seem unfavourable for their secretion because sugars would be absent or present only in very low concentrations, and because the layers next to the wall probably have the lowest proportion of impregnating and matrical substances together with the highest concentration of cellulose microfibrils. Under these conditions there could be produced enzymes such as C_1, swelling factor, or the hypothetical "X" enzyme or enzyme complex which may be involved in the initial degradation of the lignin-cellulose complex (126). The local action of these enzymes might liberate high molecular weight compounds that would block the vascular elements in the way described for other substances. Otherwise these enzymes would be translocated to produce their effects elsewhere, possibly in the leaves. As with the pectic enzymes, systematic studies with low concentrations of purified and well characterized cellulases are now needed.

Finally, it may be mentioned that a cellulase which hydrolyzes soluble derivatives has now been obtained from germinating uredospores of *Puccinia graminis tritici* (119).

Conclusions

It is now known that many plant pathogens can secrete pectic and cellulolytic enzymes and there is abundant evidence that the pectic enzymes are important in a number of plant diseases. Evidence for the importance of cellulases is less complete and conclusive but this is probably because they have not been studied as much from this point of view. Unfortunately, most work with these enzymes in plant pathology in the past has stopped with the demonstration that a particular pathogen can produce them *in vitro*. Relatively few attempts have been made to characterize the enzymes and

to see if they are produced *in vivo*. Clearly, more detailed studies are now needed on the types of enzymes produced, and their effects, alone or in combination, on cell walls and on the protoplasts. Results obtained with purified preparations can then be compared with comparative analyses of healthy and diseased tissues, and in this way a picture will be slowly built up of the ways in which host tissue is degraded. It will also be necessary to take into account the action of enzymes that affect the other components of the cell wall. There is growing evidence of their importance, particularly in young cell walls, and so far such enzymes have been almost completely ignored. At present almost no evidence exists, one way or another, as to their significance.

LITERATURE CITED

1. Kerr, T., *Plant Growth Substances* (University of Wisconsin Press, Madison, Wisconsin, 476 pp., 1951)
2. Northcote, D. H., *Biol. Revs. Cambridge Phil. Soc.*, **33**, 53 (1958)
3. Preston, R. D., *The Molecular Architecture of Plant Cell Walls* (Chapman & Hall, London, England, 211 pp., 1952)
4. Frey-Wyssling, A., *Submicroscopic Morphology of Protoplasm and Its Derivatives* (Elsevier, Houston, Texas, 411 pp., 1953)
5. Frey-Wyssling, A., *Nature*, **179**, 941 (1957)
6. Belford, D. S., Myers, A., and Preston, R. D., *Nature*, **181**, 1251 (1958)
7. Roelofsen, P. A., and Kreger, D. R., *J. Exptl. Bot.*, **2**, 332 (1951)
8. Preston, R. D., *J. Exptl. Bot.*, **5**, 26 (1954)
9. Bayley, S. T., Colvin, J. R., Cooper, F. P., and Martin-Smith, C. A., *J. Biophys. Biochem. Cytol.*, **3**, 171 (1957)
10. Bayley, S. T., and Setterfield, G., *Ann. Botany (London)*, **21**, 633 (1957)
11. Beer, M., and Setterfield, G., *Am. J. Botany*, **45**, 571 (1958)
12. Scott, F. M., Hammer, K. C., Baker, E., and Bowler, E., *Am. J. Botany*, **45**, 449 (1958)
13. Setterfield, G., and Bayley, S. T., *J. Biophys. Biochem. Cytol.*, **4**, 377 (1958)
14. Bishop, C. T., Bayley, S. T., and Setterfield, G., *Plant Physiol.*, **33**, 283 (1958)
15. Ray, P. M., *Plant Physiol.*, Proceedings (1958)
16. Albersheim, P., *Plant Physiol.*, Proceedings (1958)
17. Ginzburg, B. Z., *Nature*, **181**, 398 (1958)
18. Kertesz, Z. I., *The Pectic Substances* (Interscience, New York, N.Y., London, England, 628 pp., 1951)
19. Lineweaver, H., and Jansen, E. F., *Advances in Enzymol.*, **11**, 267 (1951)
20. Hinton, C. L., *Ann. Rev. of Biochem.*, **20**, 67 (1951)
21. Demain, A. L., and Phaff, H. J., *Wallerstein Lab. Commun.*, **20**, 119 (1957)
22. McCready, R. M., and Owens, H. S., *Econ. Botany*, **8**, 29 (1954)
23. Henglein, F. A., *Modern Methods of Plant Analysis*, **II** (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 626 pp., 1955)
24. Deuel, H., and Stutz, E., *Advances in Enzymol.*, **20**, 341 (1958)
25. Brown, W., *Ann. Botany (London)*, **29**, 313 (1915)
26. Gäumann, E., and Böhni, E., *Helv. Chim. Acta*, **30**, 24 (1947)
27. Gäumann, E., and Böhni, E., *Helv. Chim. Acta*, **30**, 1591 (1947)
28. Grosclaude, C., *Compt. rend.*, **246**, 1273 (1958)

29. Lakshminarayanan, K. ,*Physiol. Plantarum,* **10,** 877 (1957)
30. Phaff, H. J., *Arch. Biochem. and Biophys.,* **13,** 67 (1947)
31. Oxford, A. E., *Nature,* **154,** 271 (1944)
32. Waggoner, P. E., and Dimond, A. E., *Phytopathology,* **45,** 79 (1955)
33. Beckman, C., *Phytopathology,* **46,** 605 (1956)
34. Ceponis, M. J., and Friedman, B. A., *Phytopathology,* **49,** 141 (1959)
35. Echandi, E., Van Gundy, S. D., and Walker, J. C., *Phytopathology,* **47,** 549 (1957)
36. Fergus, C. L., and Wharton, D. C., *Phytopathology,* **47,** 635 (1957)
37. Husain, A., and Kelman, A., *Plant Disease Rept.,* **40,** 629 (1956)
38. Husain, A., and Rich, S., *Phytopathology,* **48,** 316 (1958)
39. Scheffer, R. P., Gothoskar, S. S., Pierson, C. F., and Collins, R. P., *Phytopathology,* **46,** 83 (1956)
40. Singh, R. K., and Wood, R. K. S., *Ann. Botany (London),* **20,** 89 (1956)
41. Srivastava, D. N., Echandi, E., and Walker, J. C., *Phytopathology,* **49,** 145 (1959)
42. Wood, R. K. S., *Ann. Botany (London),* **19,** 1 (1955)
43. Smith, W. K., *J. Gen. Microbiol.,* **18,** 33 (1958)
44. Kamal, M., and Wood, R. K. S., *Ann. Appl. Biol.,* **44,** 322 (1956)
45. Gupta, S. C., *Ann. Botany (London),* **20,** 179 (1956)
46. Menon, K. P. V., *Ann. Botany (London),* **48,** 187 (1934)
47. Chona, B. L., *Ann. Botany (London),* **46,** 1033 (1932)
48. Cole, J. S., *Ann. Botany (London),* **20,** 15 (1956)
49. Fernando, M., *Ann. Botany (London),* **1,** 727 (1937)
50. Gregg, M., *Ann. Botany (London),* **16,** 235 (1952)
51. Jarvis, W. R., (Doctoral thesis, Univ. of London, London, England, 1953)
52. Akai, S., and Oishi, C., *Shokubutsu Byôgai Kenkyu,* **5,** 57 (1955)
53. Murant, A. F., and Wood, R. K. S., *Ann. Appl. Biol.,* **45,** 635 (1957)
54. Dimond, A. E., and Waggoner, P. E., *Phytopathology,* **43,** 229 (1953)
55. Gee, M., Reeve, R. M., and McCready, R. M., *J. Agr. Food Chem.,* **7,** 34 (1959)
56. McCready, R. M., and Reeve, R. M., *J. Agr. Food Chem.,* **3,** 260 (1955)
57. Reeve, R. M., *Am. J. Botany,* **46,** 241 (1959)
58. Cole, M., (Doctoral thesis, Univ. of London, London, England, 1958)
59. Wood, R. K. S., and Gupta, S. C., *Ann. Botany (London),* **22,** 309 (1958)
60. Brown, W., and Harvey, C. C., *Ann. Botany (London),* **40,** 643 (1927)
61. Green, M., *J. Pomol. Hort. Sci.,* **10,** 184 (1932)
62. Bennet-Clark, T. A., *The Chemistry and Mode of Action of Plant Growth Substances* (Academic Press Inc., New York, N.Y., 312 pp., 1956)
63. Bryan, W. H., and Newcombe, E. H., *Physiol. Plantarum,* **7,** 290 (1954)
64. Buffel, K., and Carlier, A., *Agricultura (Louvain),* **4,** 157 (1956)
65. Glasziou, K. T., and Inglis, S. D., *Australian J. Biol. Sci.,* **11,** 127 (1958)
66. Neely, W. B., Ball, C. D., Hamner, C. L., and Sell, H. M., *Plant Physiol.,* **25,** 525 (1950)
67. Orgell, W. H., *Plant Physiol.* **30,** 78 (1955)
68. Bell, T. A., and Etchells, J. L., *Appl. Microbiol.,* **4,** 196 (1956)
69. Holden, M., *Biochem, J. (London),* **47,** 415 (1950)
70. Kaji, A., *Bull. Agr. Chem. Soc. Japan,* **20,** 8 (1956)
71. Hayashi, T., *Hakkô Kôgaku Zasshi,* **36,** 246 (1958)

72. Fushtey, S. G., *Ann. Botany (London)*, **21,** 273 (1957)
73. Tribe, H. T. (Doctoral thesis, Univ. of London, London, England, 1951)
74. Tribe, H. T., *Ann. Botany (London)*, **19,** 351 (1955)
75. Chayen, J., *Nature*, **170,** 1070 (1952)
76. McColloch, R. J., and Kertesz, Z. I., *Arch. Biochem. Biophys.*, **13,** 217 (1947)
77. Rahman, M. B., and Joslyn, M. A., *Food Research*, **18,** 301 (1953)
78. Mehlitz, A., and Maass, H., *Z. Untersuch. Lebensm.*, **70,** 180 (1935)
79. Akai, S., and Oishi, C., *Chem. Abstr.*, **49,** 1158 (1955)
80. Bell, T. A., and Etchells, J. L., *Botan. Gaz.*, **119,** 192 (1958)
81. Byrde, R. J. W., *J. Hort. Sci.*, **31,** 188 (1956)
82. Byrde, R. J. W., *J. Hort. Sci.*, **32,** 227 (1957)
83. Echandi, E., and Walker, J. C., *Phytopathology*, **47,** 303 (1957)
84. Etchells, J. L., Bell, T. A., and Williams, C. F., *Food Technol.*, **12,** 204 (1958)
85. Grossman, F., *Naturwissenschaften*, **45,** 113 (1958)
86. Hathway, D. E., and Seakins, J. W. T., *Biochem. J. (London)*, **70,** 158 (1958)
87. Kieser, M. E., Pollard, A., and Sissons, D. J., *Ann. Rept. Long Ashton Res. Station, 1957*, **134** (1957)
88. Pollard, A., Kieser, M. E., and Sissons, D. J., *Chem. & Ind. (London)*, **952** (1958)
89. Ramaswamy, M. S., and Lamb, J., *J. Sci. Food Agr.*, **9,** 46 (1958)
90. Weurman, C., *Acta. Botan. Neerl.*, **2,** 107 (1953)
91. Cole, M., *Nature*, **181,** 1596 (1958)
92. Milthorpe, F. L., *Proc. Linnean Soc. N.S. Wales*, **66,** 65 (1941)
93. Fernando, M., and Stevenson, G., *Ann. Botany (London)*, **16,** 103 (1952)
94. Lapwood, D. H., *Ann. Botany (London)*, **21,** 167 (1957)
95. Murant, A. F., and Wood, R. K. S., *Ann. Appl. Biol.*, **45,** 650 (1957)
96. Henglein, F. A., and Vollmert, B., *Makromol. Chem.*, **2,** 77 (1948)
97. Ashworth, P. R., *Dissertation Abstr.*, **14,** 446 (1954)
98. Cooil, B. J., and Bonner, J., *Planta*, **48,** 696 (1957)
99. Doesburg, J. J., *J. Sci. Food Agr.*, **8,** 206 (1957)
100. Edgington, L. V., and Walker, J. C., *Phytopathology*, **48,** 324 (1958)
101. Spurr, A. R., *Am. J. Botany*, **44,** 637 (1957)
102. Stapp, C., and Hartwich, W., *Zentr. Bakteriol. Parasitenk. Abt. II*, **110,** 449 (1957)
103. Tagawa, T., and Bonner, J., *Plant Physiol.*, **32,** 207 (1957)
104. Whittington, W. J., *J. Exptl. Botany*, **10,** 93 (1959)
105. Corden, M. E., and Dimond, A. E., *Phytopathology*, **49,** 68 (1959)
106. Ordin, L., Cleland, R., and Bonner, J., *Plant Physiol.*, **32,** 216 (1957)
107. Ordin, L., Cleland, R., and Bonner, J., *Proc. Natl. Acad. Sci. U.S.*, **41,** 1023 (1955)
108. Wilson, C. M., and Skoog, F., *Physiol. Plantarum*, **7,** 80 (1954)
109. Friedman, B. A., and Ceponis, M. J., *Phytopathology*, **49,** 227 (1959)
110. Husain, A., and Kelman, A., *Phytopathology*, **48,** 377 (1958)
111. Dimond, A. E., and Husain, A., *Science*, **127,** 1059 (1958)
112. Gothoskar, S. S., Scheffer, R. P., Walker, J. C., and Stahmann, M. A., *Phytopathology*, **45,** 381 (1955)
113. Pierson, C. F., Gothoskar, S. S., Walker, J. C., and Stahmann, M. A. *Phytopathology*, **45,** 524 (1955)
114. Winstead, N. N., and Walker, J. C., *Phytopathology*, **44,** 153 (1954)

115. Ludwig, R. A., *Macdonald Coll. Tech. Bull.*, **20** (1952)
116. Collins, R. P., and Scheffer, R. P., *Phytopathology*, **48**, 349 (1958)
117. McDonnell, K., *Nature*, **182**, 1025 (1958)
118. Brooks, F. T., and Brenchley, G. H., *New Phytologist*, **30**, 128 (1931)
119. Sumere, C. F., Van-Sumere-De Preter, C., and Ledingham, G. A., *Can. J. Microbiol.*, **3**, 761 (1957)
120. Fahraeus, G., and Ljunggren, H., *Physiol. Plantarum*, **12**, 145 (1959)
121. Siu, R. G. H., *Microbial Decomposition of Cellulose* (Reinhold Pub. Corp., New York, N.Y., 531 pp., 1951)
122. Siu, R. G. H., and Reese, E. T., *Botan. Rev.*, **19**, 377 (1953)
123. Greathouse, G. A., and Wessel, C. J., *Deterioration of Materials* (Reinhold Pub. Corp., New York, N.Y., 835 pp., 1954)
124. Jermyn, M. A., *Modern Methods of Plant Analysis*, **II** (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 629 pp., 1955)
125. Reese, E. T., *Appl. Microbiol.*, **4**, 39 (1956)
126. Cowling, E. B., *Rept. Forest Prod. Lab., Madison*, **2116**, 1–26 (1958)
127. Thomas, R., and Whitaker, D. R., *Nature*, **181**, 715 (1958)
128. Whitaker, D. R., *Arch. Biochem. and Biophys.*, **43**, 253 (1953)
129. Whitaker, D. R., *Can. J. Biochem. and Physiol.*, **35**, 733 (1957)
130. Aitken, R. A., Eddy, B. P., Ingram, M., and Weurman, C., *Biochem. J. (London)*, **64**, 63 (1956)
131. Kooiman, P., *Enzymologia*, **18**, 371 (1957)
132. Sison, B. C., Schubert, W. J., and Nord, F. F., *Arch. Biochem. Biophys.*, **75**, 260 (1958)
133. Grimes, R. M., Duncan, C. W., and Hoppert, C. A., *Arch. Biochem. Biophys.*, **68**, 412 (1957)
134. Hash, J. H., and King, K. W., *J. Biol. Chem.*, **232**, 381 (1958)
135. Miller, G. L., and Blum, R., *J. Biol. Chem.*, **218**, 131 (1956)
136. Reese, E. T., and Levinson, H. S., *Physiol. Plantarum*, **5**, 345 (1952)
137. Reese, E. T., and Gilligan, W., *Arch. Biochem. and Biophys.*, **45**, 74 (1953)
138. Reese, E. T., Segal, L., and Tripp, V. W., *Textile Research J.*, **27**, 626 (1957)
139. Marsh, P. B., *Plant Disease Reptr.*, **37**, 71 (1953)
140. Reese, E. T., and Gilligan, W., *Textile Research J.*, **24**, 633 (1954)
141. Siu, R. G. H., and Sinden, J. W., *Am. J. Botany*, **38**, 284 (1951)
142. Akai, S., *Shokubutsu Byôgai Kenkyu*, **4**, 64 (1951)
143. Amman, A., *Phytopathol. Z.*, **18**, 416 (1952)
144. Goto, M., and Okabe, N., *Nature*, **182**, 1516 (1958)
145. Husain, A., *Phytopathology*, **48**, 338 (1958)
146. Husain, A., and Dimond, A. E., *Phytopathology*, **48**, 263 (1958)
147. Klug, R. J., and Threinen, J. T., *Phytopathology*, **47**, 21 (1957)
148. Kohlmeyer, J., *Phytopathol. Z.*, **27**, 147 (1956)
149. Talboys, P. W., *Brit. Mycol. Soc. Trans.*, **41**, 242 (1958)

PHYSICS AND CHEMISTRY OF PROTOPLASMIC STREAMING[1,2]

By N. Kamiya

Department of Biology, Faculty of Science, Osaka University, Osaka, Japan

In spite of its long history of research, protoplasmic streaming still has a great many obscure features both in its physiological nature and in its mechanism. As the author has recently discussed this complicated and intriguing phenomenon and surveyed the past activities rather fully in a monograph (1), the scope of the following review will be restricted to some selected topics in recent literature.

Among the various types of protoplasmic streaming, what is known as rotation in plant cells and shuttle streaming in plasmodia of myxomycetes was the subject of extensive study recently. However, the streaming in plant cells having cell walls (often referred to as cyclosis) and the streaming in the slime mold, which, like that in amoeba involves concomitant changes in the external form of the protoplast and is usually accompanied by locomotion, differ not only in their modes of flow, but also in research methods used to investigate them. Thus it seems pertinent for this review to consider the two types of motion separately.

Protoplasmic Streaming in Plant Cells (Cyclosis)

Site of the force causing rotational streaming.—What is usually called rotation designates motion in which the protoplasm circulates close to the wall of a cell in a band-like form. Since this movement consists of a closed circuit, it has, so to speak, no head or tail. One of the basic problems concerning the mechanism of this elusive motion is that of determining the site of the motive force responsible for the streaming. The problem has been unsettled for a long time and there have been diverse opinions none of which have had strong experimental support. Recent evidence, however, increasingly enhances the importance of the role of sol-gel interfaces for the mechanism of rotational streaming.

One experimental approach to this problem is to investigate the precise velocity distribution in different layers of the streaming protoplasm in suitable material. It was reported by Kamiya & Kuroda (2) that in the rhizoid and "leaf" cells of *Nitella*, both of which show a typical rotational streaming, the whole endoplasm flows with a nearly equal rate, there being no appreciable shear inside. It is, as it were, just sliding as a whole on the inner surface of the cortical layer. A similar situation prevails in internodal cells.

[1] The survey of literature pertaining to this review was completed August 1959.

[2] The following abbreviations will be used: AMP (adenosine monophosphate); DPG (dynamoplasmogram); PCMB (*p*-chloromercuribenzoate); SH (sulfhydryl).

The same authors observed a rather vigorous streaming in certain internodal cells of *Nitella* which were lacking visible vacuoles. Such cells were obtained by an operation combining centrifugation and subsequent ligation as was tried by Hayashi (3). As cells with active protoplasmic streaming generally have well-developed vacuoles, some authors have long believed that the existence of vacuoles is an indispensable condition for such streaming. But now that it has been shown explicitly that protoplasmic streaming actively occurs in cells experimentally deprived of vacuoles, it is clear that the presence of the large vacuole is not a prerequisite for the occurrence of streaming. A further important fact revealed by streaming in a cell without vacuoles is that the rate of streaming is highest in the outermost layer of protoplasmic sol, i.e., in direct proximity to the cortical gel layer, and lessens closer to the central axis of the cell, where no flow is observed. In this case the velocity profile of the endoplasmic flow neatly coincides with that of a fluid between two semicylindrical walls that are made to slide past each other in a longitudinal direction. The logical conclusion derived from this fact is that in the *Nitella* cell a parallel displacement force is generated at the interfacial region between the endoplasm and the cortical gel layer in such a way that the outer edge of the endoplasm tends to be shifted along the inner surface of the cortical gel layer in one direction on one longitudinal half of the cell and in the opposite direction on the other half. This active shearing force working in parallel to the interface is responsible for the rotation of the endoplasm in the cell of *Nitella*.

There are also various evidences in the older literature supporting the view that the organized cortical gel surface is indispensable for maintaining protoplasmic flow in the cells of the family *Characeae*. For instance, if the cortical gel layer of *Nitella* or *Chara* cells is injured locally, the protoplasmic flow no longer occurs at the spot of injury, but is diverted around it (4, 5, 6). Or, if the cortical gel of the cell is partially removed by strong centrifugation, the flow no longer occurs where the cortical gel was exfoliated. The observations of Breckheimer-Beyrich (7) also point out the importance of the cortical plasmagel for the occurrence of streaming. She centrifuged the internodal cell of *Nitella flexilis* and observed with the centrifuge-microscope that the endoplasm collected at the centrifugal end of the cell in a tumbled mass streamed back normally after centrifugation. It should be stressed that in this case the course and direction of flow were always the same as before the cell was centrifuged. Breckheimer-Beyrich interprets this as being attributable to some function of the cortical gel in guiding the endoplasm. Observations on the behavior of isolated drops of protoplasm, as well as the recent discovery by Jarosch (8, 9, 10, 11), of moving fibrils in protoplasm, to which we shall return in a later section, also focus on the same point, i.e., the essential role played by the sol-gel interface in protoplasmic streaming.

Opinions about the site of the motive force, however, have not always

been unanimous. Some authors suggested that the motive force driving the protoplasm resides in the flowing protoplasm itself (12, 13, 14, 15). A recent advocate of this theory is Kishimoto (16) who emphasizes the importance of submicroscopic oscillation of the networks formed by the contractile proteins in the endoplasm and believes that the endoplasm can move with its own power, independent of the cortical gel. When discussing the mechanism of streaming from this viewpoint one must keep in mind that the transmission of contractile waves is quite different from the bodily movement of the medium (endoplasm). Hence, any view postulating the site of the motive force in the streaming material itself must clarify, even in a simplified logical model, how the internal force can bring about the one-directional mass streaming. With the present status of our knowledge it is difficult to find evidences favoring the view that the motive force is inherent in the stream itself. Taking into consideration all of the experimental facts so far known [for details see Kamiya (1)] the most natural and adequate conclusion concerning the site of the motive force of rotation, at least in the cells of the *Characeae,* would be that it is at the interface between the organized cortical gel layer and the outer edge of the endoplasm.

Behavior of the isolated endoplasmic drop.—According to the above conclusion it follows that protoplasmic streaming is maintained only when there is a cortical gel layer present. In other words, the endoplasm alone is not capable of producing the flow. To see whether or not this prediction is valid, Kamiya & Kuroda (17) isolated "pure" endoplasmic drops from an internodal cell of *Nitella,* using a new method which they developed.

The naked endoplasmic drops obtained by them can survive more than 24 hr. and sometimes even more than five days *in vitro* (18). In such drops of endoplasm can be seen a lively Brownian movement, but no mass flow of the endoplasm (19). One phenomenon to be observed here however, is the behavior of the nuclei and choloroplasts contained in this drop. Though the endoplasm itself exhibits no mass streaming, each of the nuclei and chloroplasts turns very actively on its own axis. Recently Fetzmann (20) ascertained in *Chara foetida* that the older the internodal cell from which the nuclei are obtained the faster are the rotations of the nuclei. The swifter nuclei make a full rotation in less than one second. When a number of chloroplasts are linked in a row, each does not rotate around its own axis, but all of them, remaining in a row, move about in the endoplasmic drop (21, 9). By careful observation, it is possible to notice that in the very thin layer of endoplasm immediately adjacent to the chloroplasts, a streaming occurs in the direction opposite to that of their rotation. This phenomenon is also found with the rotation of nuclei. The occurrence of such a counter-streamlet was described by many authors in the past [cf. Kamiya (1)] and recently stressed by Jarosch (9, 11). In any case, this is a further proof for the existence of a counter-shifting force between sol and gel.

In protoplasmic streaming within the cell the cortical gel protoplasm is fixed, so that the shearing force generated at the interface between it

and the endoplasmic sol manifests itself as a streaming of the endoplasm. In the case of nuclei and chloroplasts, however, the gel phase is freely suspended in the sol phase of the endoplasm. Therefore, if the site of the force generating a shift occurs at the surface of these organelles, movement and rotation of the gel phase develops instead of streaming of the sol phase. No matter which of the two moves, the basic mechanism seems the same. The fact that nuclei and chloroplasts rotate around their own axes must be attributable to the presence somewhere on their surface of a structure probably having the same function as the cortical plasm. The moving protoplasmic fibrils which we are going to consider next may have an important bearing on consideration of such a structure.

Moving fibrils in the protoplasm.—We are still ignorant about the nature of the shifting force at the interface between cortical gel and endoplasm. However, recent observations of Jarosch (8, 9, 10, 11) on the behavior of the protoplasmic fibrils in an isolated drop of protoplasm from internodal cells of the *Characeae* are very instructive in this connection. The present author could also confirm in *Nitella* many of Jarosch's observations.

When a protoplasmic drop that has been mechanically squeezed out from the cells of *Nitella* or *Chara* is observed with bright field illumination, many granules, nuclei, and chloroplasts which show Brownian movement can be seen. Careful observation of these with an oil immersion lens, under dark field illumination, makes it barely possible to perceive moving fibrillar structures which could not be seen in the bright field. These structures take on various forms; fibrils waving like flagella; those with both ends connected to form round or oval loops; and those with a part of them stretched in a straight line like a bow, and often consisting only of rectilinear parts forming equilateral polygons. The fibrils themselves are barely visible but their presence may be determined by microsomes attached to them. All of these fibrils move actively, the string-like forms moving with a waving motion, and the circular and polygonal forms rotating on their own axes. Interestingly enough, in this case too, the minute granules which are in contact with the fibrils are always pushed in a direction opposite to that of the movement of the fibrils, showing a relationship of counter-shifting. This noteworthy fact is similar to the phenomena mentioned above.

These protoplasmic fibrils, which can only be perceived through ultramicroscopy, actually consist of still finer fibrils that seem always to be made anew and to disappear again. One can observe in the dark field new fibrillar structures which, faintly glimmering, emerge unnoticed, but move so fast that it is sometimes difficult to distinguish them. These new fibrils disappear after 10 to 15 min. When they begin to vanish, one can just observe them becoming divided into finer fibrils.

If many of these protoplasmic fibrils become united into thick bundles, their forward motion is gradually checked and they eventually show a wave-like movement similar to the beating of flagella. When these bundles be-

come divided into finer fibrils, each of the fibrils ceases its wave-like motion and begins to move in a straight line, some of the fibrils going forward and some backward. In fibrils that do not form loops, the ends are never visible. It may be that the bundles are loose and tufty at the ends and cannot be resolved by ordinary microscopy.

In short, we see that within a drop of cytoplasm fine fibrils are being continually gathered and dispersed and moving structures are repeatedly formed and disintegrated. As was said before, the movements of these fibrils seem to be associated with a mechanism of counter-shifting with the immediately adjacent milieu. Setting aside the question of the nature of the fibrils, it can be said that, if such fibrillar structures are attached to the surface of chloroplasts and nuclei, a mechanism would be present for making these organelles rotate around their own axes; and if such fibrils were arranged in parallel on the interior of the cortical protoplasm, a motive force of shifting could be generated along the interface, causing the endoplasm to flow.

Measurement of the motive force of rotational streaming.—Although we do not know the physical and chemical nature of the shifting force generated at the cortical gel—endoplasm interface, it is reasonably certain that this force is responsible for rotational streaming, at least in the cases of *Nitella* and *Chara* cells. Recently an attempt was made by Kamiya & Kuroda (22) to measure the absolute magnitude of this force, or the motive force responsible for the rotational streaming. The dimension of this force is expressed by $[ML^{-1}\ T^{-2}]$, which is similar to pressure, but differs from pressure in that the force is exerted parallel to the interface.

The principle of the measurement is as follows: when an internodal cell is centrifuged at a moderate speed with the force directed along its longitudinal axis, it may be seen by centrifuge-microscope observation that the streaming toward the centrifugal end of the cell is accelerated while the streaming toward the centripetal end of the cell is retarded. One can determine without difficulty the speed of centrifuge rotation at which the endoplasm streaming toward the centripetal end is brought to a standstill. This centrifugal acceleration, which was referred to as "balance-acceleration" by Kamiya and Kuroda, varies usually between 200 × g and 800 × g according to the experimental conditions [cf. Hayashi (23)]. Kamiya & Kuroda (22) calculated the motive force from the following formula: $F = Ad(D_p - D_s)\alpha$; where α represents the balance-acceleration, d the thickness of the endoplasmic layer when the outermost endoplasm, adjacent to the cortical gel layer, is kept immobile, A the area of the interface between cortical gel and endoplasm where shifting occurs, and D_p and D_s densities of endoplasm and cell sap, respectively. Fortunately, there are recent data of the same authors available concerning D_p and D_s (24). According to them $D_p - D_s$ can be regarded as 0.004 gm./cm^3. The quantities α and d were determined directly by means of a centrifuge-microscope. Calculations based on the above measurements revealed that the force in question

is as small as 1 to 2 dynes/cm². This is the first time that a definite figure has been presented as the absolute value of the motive force of rotation.

ATP level, ATP-dephosphorylating activity, and ATP-sensitive proteins.—Recently Hatano & Nakajima (25) assayed the ADP and ATP content in *Nitella* by chromatographic analysis using ion exchange resins after the method of Cohn & Carter (26). Energy-rich phosphate compounds were first extracted with barium salts. According to Hatano & Nakajima, *Nitella* contains about 0.04 μmoles of ATP and 0.04 μmoles of ADP per gram wet weight. This means that the ATP content in *Nitella* is about one-tenth of that of the *Physarum* plasmodium (cf. later section). But considering that the protoplasm in the *Nitella* cell is only about one-tenth the cell volume and the remaining part is occupied mostly by a big vacuole, it may be said that the average ATP content per unit weight of *Nitella* protoplasm is almost the same as of the *Physarum* plasmodium. These authors further demonstrated ATP-dephosphorylating activity of the apyrase type of KCl extracts obtained from fresh *Nitella* cells and from glycerine-treated *Nitella* cells. Hence it can be stated that some protein or proteins are present in *Nitella* cells that have the following characteristic properties: they are insoluble in water, but soluble in KCl solution of high concentration, and they have ATP-dephosphorylating activity.

Takata (27) applied the procedure of glycerination to *Acetabularia* cells and observed that a transient motion arose in a protoplasmic mass in a state of gel when 1×10^{-3} *M* ATP was added to it together with 1×10^{-3} *M* $MgCl_2$. Naturally a motion of this kind occurring in glycerinated protoplasm in the gel state is not comparable with the flow in the living cell, but it is interesting that such a movement does not occur when the glycerinated cell is treated with a high concentration (above 0.3 *M*) of KCl solution. These recently obtained facts suggest that some actomyosin-like protein may be present in cells of plants such as *Nitella* and *Acetabularia*, although isolation of actomyosin-like proteins from these cells has been so far unsuccessful.

Effect of ATP on the streaming in plant cells.—The effect of ATP on protoplasmic streaming in living plant cells that have cell walls has been studied but little. The work of Takata (27) and Sandan (28) is along this line. The effect of ATP on streaming in the slime mold will be discussed later.

Takata (27) observed that streaming in the *Acetabularia* stalk is stimulated to four times normal when 5×10^{-4} *M* ATP (dissolved in sea water) is applied from the outside. A characteristic effect in this case is that the stimulation caused by ATP is only transient; the rate returns to the original level in 10 min. or so. We do not know why the effect does not last longer. If the concentration of ATP is raised to 2×10^{-3} *M*, the rate of movement first increases but soon ceases to flow usually 4 to 5 min. after the reagent is administered. Neither muscle adenylic acid nor inorganic pyrophosphate are potent in causing the stimulation, and hence this reaction, if temporary, may be ATP-specific.

Sandan (28) studied the effect of ATP in various concentrations on protoplasmic streaming in internodal cells of *Nitella flexilis*. The concentration range of ATP that stimulates the flow is rather narrow. The maximum stimulating effect was found with 1×10^{-3} *M* ATP. The rate of streaming was accelerated 15 per cent above the control at this concentration. This acceleration became visible about 15 min. after the reagent was applied and the accelerated rate was maintained constant for more than 50 min. When ATP was removed, the rate of flow dropped to the initial level within 10 min. Concentrations below 1×10^{-4} had no effect on the rate, while 1×10^{-2} *M* ATP gradually retarded the streaming until it had stopped completely within 70 min. The flow did not recover after 1×10^{-2} *M* ATP was removed. Concentrations higher than 1×10^{-2} *M* stop the streaming quickly and permanently.

SH-reagents.—Abe (29) observed the behavior of protoplasmic streaming in cells of *Nitella, Elodea, Hydrocharis,* and *Tradescantia* under the influence of *p*-chloromercuribenzoate (PCMB) which blocks SH groups with high specificity. This reagent, being poorly soluble at neutral pH, was dissolved in 10^{-2} *M* NaOH and then neutralized with HCl. Thus a cell in 10^{-2} *M* NaCl served as the control.

The effect of PCMB is very conspicuous and remarkable. With 10^{-3} *M* PCMB the flow of protoplasm stops completely within a few minutes, sometimes even within a few seconds. There is no sign of pathological changes in the protoplasm except that it is at a standstill. It is to be stressed that recovery of streaming is never obtained merely by removing the reagent from the medium. The streaming is restored completely or fairly well, however, if the inhibitor medium is replaced by a medium containing a sufficient amount of cysteine (10^{-2} *M*). In the case of *Nitella,* the flow starts again locally in a few minutes after PCMB is replaced with 10^{-2} *M* cysteine.

Kishimoto and Akabori (30) also observed the effect of 10^{-4} *M* PCMB (dissolved in tap water, adjusted to pH 6.8, total Na content lower than .002 *M*) on *Nitella.* According to them velocity decrease in the presence of PCMB is progressive until the flow stops completely in an hour or so. If 10^{-3} *M* cysteine (pH 6.7) is substituted for PCMB, however, the flow tends to recover. Tap water or other salt solutions are not effective in restoring the flow.

Also in *Elodea* the rate decreases precipitously in the presence of 10^{-3} *M* PCMB until it stops completely within a few minutes (29). If 10^{-2} *M* cysteine is added 10 min. after the streaming has stopped completely, streaming is resumed until full recovery is attained after 40 min. Again, there is no sign of recovery if PCMB is simply removed from the medium. This is quite in conformity with the observation of Smirnova (31) who administered $CdCl_2$ instead of PCMB to *Elodea* leaves and ascertained that streaming was resumed following subsequent application of cysteine.

The sensitivity of protoplasmic streaming to a SH-reagent differs according to the material. The streaming in the root hair of *Hydrocharis* is highly sensitive to PCMB. It stops completely within 6 to 10 sec. in 10^{-4} *M*

PCMB. The reaction is again reversible with the application of 10^{-2} *M* cysteine. A similar phenomenon is also observable in the stamen hair of *Tradescantia* (*29*).

All of the above results indicate that the protoplasmic streaming in some of the representative materials is brought to a standstill with PCMB or $CdCl_2$ at suitable concentrations, a stop from which it recovers through subsequent application of cysteine. As it is known that PCMB is specifically bound to SH groups and that this binding is released in the presence of compounds of low molecular weight and having a SH group, it is reasonable to assume that the cessation and recovery of flow mentioned above are closely related to the blocking and liberation of SH groups in the protoplasm. The behavior of protoplasmic streaming under the influence of SH-reagents supports the view that cyclic conversion of —S—S— to —SH HS— plays an important role in the mechanism of protoplasmic streaming.

Active shifting in rotational streaming and in other biological motions. —Although we are still totally ignorant about the mechanism of active shifting at the sol-gel interface, it is very interesting that a recent theory of muscle contraction also postulates a sliding mechanism. Huxley and Hanson showed in many of their papers [for references see (*32*, *33*, 34)] that in the myofibrils a double array of filaments slide into each other during contraction. According to these authors sliding occurs between actin filaments and myosin filaments. Weber (34) elaborated a theory explaining a possible cycle of chemical reactions leading to a shifting of the actin filaments past the molecules of myosin-A filaments with simultaneous splitting of inorganic phosphate from ATP. In Weber's diagram the SH groups of myosin play an important part in the sliding mechanism. Noteworthy in this connection is the fact that the protoplasmic streaming in plant cells is very sensitive to SH-reagents, especially to PCMB. At any rate the line of thought expressed by Weber on muscle contraction is also a suggestive hypothetical model of the molecular mechanism of sliding involved in the protoplasmic streaming.

Interesting facts were pointed out recently by Jahn & Rinaldi (35) about the protoplasmic motion in reticulopodia of the foraminiferan, *Allogromia laticollaris*. This motion, which is called filament streaming, is always a two-directional movement of two threads of plasmagel. They ascribed the cause of this movement to "active shearing or parallel displacement forces located between the adjacent surfaces of the two gel filaments, acting longitudinally and oppositely from one filament to the other." This idea is basically in accord with the conclusion reached for *Nitella* cells that the motive force of the rotational streaming is the active shifting force exerted tangentially at the cortical gel-endoplasm interface (2). Jarosch's observation on the moving protoplasmic fibrils (8, 9, 10, 11; see also foregoing pages) is also in conformity with Jahn's theory on the filament streaming.

Jarosch (36) later extended the idea of the counter-shifting force produced between the protoplasmic fibrils and the surrounding milieu to the

explanation of the mechanism of motions of various lower organisms such as desmids, penate diatoms, *Oscillatoria,* etc., having no special motile apparatus. At present the weight of evidence favors the view, among others, that the active shifting force is the common basic mechanism of diverse biological motions.

Protoplasmic Streaming in Myxomycete Plasmodia

Recently the physics and chemistry of protoplasmic streaming have been extensively studied in myxomycete plasmodia. It was Seifriz who first used *Physarum polycephalum* and played a prominent part in leading recent progress and popularization in the study of protoplasmic streaming with this material (37).

Physarum polycephalum can be grown easily and abundantly on moist filter paper with rolled oats after the method of Camp (38). As this plasmodium grows rapidly, a simple culture can provide a great quantity of protoplasm for experiment. Thus it is a material highly favourable for carrying out experimental work on protoplasm. On the general morphology, physiology, and culture of myxomycetes and their movement, there are reports by Howard (39), Jahn (40), Mangenot (41), Camp (42), Cohen (43, 44), Dalleux (45), Martin (46), Lewis (47), Seifriz (48), Sobels (49), Andresen & Pollock (50), Johnson & Moos (51), Kamiya (52), etc.

General features.—The protoplasmic streaming of the slime mold is very active, the velocity and intensity of flow being much greater than in other organisms. As the slime mold has no cell membrane, its external form changes as protoplasmic streaming occurs in its interior. Moreover, it has the striking characteristic of reversing the direction of flow with a regular cycle of 2 to 3 min.

When we observe a strand of protoplasm of the slime mold under the microscope, we can see sol protoplasm flowing vigorously in the tube formed by a wall of gel protoplasm. The streaming is fastest at the central axial part, its velocity distribution being in the form of a flat-headed parabola (53). This resembles the case of solutions with structural viscosity flowing in capillary tubes. The shape of the velocity profile of the streaming in a plasmodial capillary tube is the same no matter whether the flow takes place under natural conditions according to the autonomic scheme of the plasmodium or is caused artificially by a pressure applied from the outside at the moment of reversal when the natural flow temporarily stops (54).

The protoplasmic streaming is always being either accelerated or decelerated, its changes with time differing with the locus even in the same myxomycete. A continuous record of the ever-changing velocity of movement may be obtained by photographic registration of shadows of the streaming protoplasm on a strip of film moving at constant speed at a right angle to it (1, 55). By means of measuring inclinations of shadow lines thus obtained, Kamiya (55) found that the streaming velocity of *Physarum*

polycephalum reached 1.35 mm./sec. at its highest point. This is the highest value presently known for protoplasmic streaming.

The gradual creeping locomotion of the slime mold on the substrate is due to the fact that there is, more or less, a difference in the volume of protoplasm carried in opposite directions through the rhythmic back and forth movements. If we make a continuous record of the movement of protoplasm in the slime mold by means of a double-chamber volumeter, it is possible to represent on one curve the volume of protoplasm carried by periodic back and forth streamings and the duration of each of the streamings (56). One of the curves presented by Kamiya (56) showing the locomotion of the organism revealed that more than 4 $mm.^3$ of protoplasm is sometimes shifted in one direction during streaming. No close relationship is found between the streaming duration and the volume of protoplasm transported. The intensity of the flow, which may be defined as the volume of protoplasm transported per unit time, can also be computed from the transport-volume curve by obtaining its slope. The maximum intensity of flow was shown to be 6×10^{-3} µl./sec.

Motive force responsible for the protoplasmic streaming in myxomycete plasmodia.—Measurement of the rate and intensity of streaming is important for presenting a quantitative account of protoplasmic streaming. Streaming is itself the final visible product of protoplasmic activities however, and is greatly influenced by other factors besides the motive force of the streaming, such as changes in viscosity. In the case of a slime mold the width of the streaming channel is another factor which may modify the rate of flow. Therefore, in order to come closer to an understanding of the causes of a change in the streaming rate occurring under certain conditions, we have to determine whether such a change is due to a change in the motive force or to changes in other factors such as viscosity, etc. As this question cannot be settled on the basis of past experiments it is difficult to interpret many experimental data obtained on the streaming rate. In order to overcome this difficulty, there is no other way but to measure directly the motive force of the streaming. The material making this measurement possible for the first time was the myxomycete plasmodium (57, 58, 59).

As protoplasmic streaming in the slime mold is closely related to changes in its external form, the speed and direction of the flow can be easily changed by giving a local pressure difference to the plasmodium. This characteristic has made measurement of the motive force of the streaming possible. The slime mold is placed in a double chamber which is divided into two compartments; a pressure difference between the two compartments just sufficient to stop the streaming within the protoplasmic tube penetrating the partition is used as a measure of the motive force of the streaming. This pressure was called "balance-pressure" and the method was called "double-chamber method." The double-chamber method enabled continuous measurement of the motive force ("dynamoplasmometry") and made it possible to

obtain new information on many aspects of the dynamic activities of the protoplasm.

Dynamoplasmogram (DPG).—As the motive force is ever changing in the living plasmodium, the balance-pressure must also be changed in a parallel fashion so that the protoplasm in the capillary is kept at a standstill. By measuring the balance-pressure without interruption, we can follow spontaneous changes in the motive force with time. The periodic patterns of the motive force curve are rich in variety. In some cases the wave form is very regular and almost sinusoidal. Often the waves increase and decrease their amplitude of the waves with regular intervals as in the case of beat waves. In some other cases a peculiar wave pattern is repeated over several waves. In still another case a wave with a characteristic form reappears with slight modifications in successive waves. Exactly speaking, the dynamoplasmogram patterns are all different according to the specimens used. These facts are explained by Kamiya as being due to the coexistence of rhythms having different periods in one and the same plasmodium (58, 59). In other words a plasmodium is polyrhythmic.

At any event, the double-chamber method gave us a unique opportunity to test the effects of various physical and chemical agents on the production of the motive force, and it opened up a new field of research in the physiological studies of protoplasmic streaming. Later an apparatus was constructed by Kamiya, Abe & Nakajima (60) which facilitated measuring both the motive force for the protoplasmic flow in the plasmodium (*Physarum polycephalum*) and simultaneously the respiration of the same plasmodium. A dumbbell-shaped plasmodium was placed in a double chamber so that the motive force could be measured by means of pressure difference between the two compartments, a pressure difference just sufficient to stop the flow in the protoplasmic strand between the two compartments. The construction of the apparatus is such that it works also as a volumetric respirometer of a differential type. It was shown that the rate of oxygen uptake in the normal material is constant, irrespective of the spontaneous change in the amplitude of the motive force.

Contractile proteins.—In order to elucidate protoplasmic streaming we must understand the mechanism through which the chemical energy is converted into mechanical energy. In this connection it is very interesting that the presence of an ATP-sensitive contractile protein which has basically the same nature as muscle myosin-B (actomyosin) has been found in the slime mold. This protein, like muscle myosin-B, changes its physical characteristics conspicuously under the effect of ATP and has the property of showing superprecipitation in the presence of ATP and 0.1 *M* KCl (61, 62). That such a protein is present in the slime mold was first shown by Loewy (63). Later Ts'o *et al.* (64, 65, 66, 67) and Nakajima (61, 62, 68) conducted detailed studies on the protein extracted from the slime mold, a protein having the same characteristics as myosin-B. All of these authors used

P. polycephalum for experimental material. Qualitatively speaking, the response of this protein to ATP is of the same nature as that of muscle myosin-B. Its sensitivity to ATP is nearly equal to that of smooth muscle myosin-B and much lower than that of myosin-B from skeletal muscles (61, 62).

Ts'o, Eggman & Vinograd (65), who called this ATP-sensitive protein "myxomyosin," made extensive studies on its physico-chemical properties by viscosity, sedimentation, birefringence, and electron microscope measurements. It was shown that the myxomyosin molecule is a long rigid rod with an average length of 4000 to 5000 A and diameter of 70 A. The molecular weight was estimated to be 6,000,000 on the basis of viscosity and sedimentation data. It is concluded that ribonucleic acid is contained in the active concentrate obtained by salting out (65), but it is believed not to be essential to the interaction with ATP (67).

Nakajima's data show that ATPase activity of plasmodial myosin-B (myxomyosin) is 6.6 μg. P/min./mg.N (K^+: 0.5 *M*, pH 6.6, 28°C.) AMP and inorganic pyrophosphate are not hydrolysed by plasmodial myosin-B. Further, the ATPase activity of myosin-B is stimulated to nearly 7 times the control value by Ca^{++} and is inhibited by 30 per cent by Mg^{++}. In addition to the effect of K^+ concentration, the antagonistic action between Ca^{++} and Mg^{++}, the effects of pH, 2,4-dinitrophenol, SH-reagents, ethylenediaminetetraacetic acid, etc. on ATPase activity of plasmodial myosin-B were investigated. They showed that plasmodial myosin-B had a far-reaching similarity to muscle myosin-B in enzymatic characteristics. Thus it has been firmly established that myxomycete plasmodium contains a protein that is basically similar to muscle myosin-B.

ATP-level of the myxomycete plasmodium.—It has been found by means of the double-chamber method that when ATP is given externally (69) or when 10^{-3} *M* ATP of one-tenth the volume of the plasmodium is injected with a fine capillary into the slime mold (70), the motive force increases conspicuously. The motive force of streaming becomes greatest when the ATP content of the mold is made higher than the normal level by about 10^{-4} *M*. When the ATP level becomes still higher, the motive force of the streaming begins to decrease. AMP and inorganic pyrophosphate have no effect at all. Those experiments support the hypothesis that ATP is the direct source of energy for streaming.

The direct quantitative measurement of ATP contained in the protoplasm of the slime mold was conducted recently by Hatano & Takeuchi (71). When they extracted the acid-soluble phosphate from the slime mold by means of trichloroacetic acid, eliminated its polysaccharides with 95 per cent ethanol, and separated this extract with barium salts into two fractions; "barium-soluble" and "barium-insoluble"; the greater part of the acid-soluble energy-rich phosphate compounds was found in the barium-insoluble fraction. The neutral solution of this fraction, just like the ATP obtained from muscle, conspicuously contracts the glycerol-extracted muscle

of the rabbit. This barium-insoluble fraction is further separated into ATP and ADP by means of chromatographic techniques using ion exchange resin (IRA 400). It is found that the protoplasm of the slime mold contains about 2.5 μmoles inorganic phosphate, 0.2 μmoles of ADP, and 0.4 μmoles of ATP per gram wet weight under normal conditions.

Energetics of protoplasmic streaming.—Summing up what has been stated concerning the myxomycete plasmodium, there is present a contractile protein having properties similar to those of myosin-B from muscle; the average content of ATP is 4×10^{-4} *M;* when ATP is given to the slime mold from without or injected within by means of a micropipette, the force of streaming increases. From these facts an assumption may be derived that the mechanochemical system participating in the protoplasmic streaming of the slime mold is the ATP-actomyosin system as is the case for muscle.

Even though the energy for streaming is provided by ATP, the energy source necessary for the synthesis of ATP must be sought in respiration or fermentation. What is the relation then between the force of streaming and these energy-liberating reactions?

That the protoplasmic flow in *Physarum* is rather insensitive to anaerobiosis or to cyanide was pointed out by Allen & Price (72) and Loewy (73). When Kamiya, Nakajima & Abe investigated the generation of the motive force of streaming under various experimental conditions by means of the ordinary double-chamber method (69) or with the method of simultaneous measurement of respiration and the force of streaming (18, 74, 75), they found that the force of streaming does not decrease, but on the contrary tends to increase under conditions suppressing respiration (low oxygen tension, HCN or KCN, CO). These results are in agreement with those of Ohta (76, 77) who also measured the motive force of flow by means of the double-chamber method and investigated the effect of various chemical agents (modified oxygen tensions, HCN, CO, chloroform) on this flow.

On the contrary, under conditions inhibiting fermentation (monoiodoacetate, NaF), the generation of the force of streaming is strongly suppressed at a concentration that does not inhibit aerobic respiration (69). Simultaneous measurement of respiration and the motive force, under the influence of 4×10^{-4} *M* monoiodoacetate, indicates that respiration does not change at all before and after applying the reagent, the Q_{O_2} being 8.0 ml./mg./hr. in both, but the average amplitude of the motive force is decreased by about one-half (78). Since monoiodoacetate has no effect whatever on the ATPase activity of myosin-B of the slime mold (61, 62), the decrease in the force of streaming caused by monoiodoacetate must not be caused by the suppression of ATP consumption but rather by the inhibition of its production.

When the slime mold is treated with monoiodoacetate or NaF, the force of streaming decreases immediately (69). This can be well understood when we consider that phosphagens such as phosphocreatine or phosphoarginine are not detected in the protoplasm of the slime mold (71). With 2,4-dinitro-

phenol the generation of the force of streaming is inhibited with or without oxygen present (69). Therefore the cause of the inhibition in this case does not seem to be related to the uncoupling of phosphorylation from aerobic respiration.

In short, the force of streaming increases when respiration is inhibited and decreases under conditions that inhibit glycolysis. All of the past experiments performed by Kamiya and his collaborators have shown that this relationship holds true qualitatively almost without exception. This fact shows that the energy source for the generation of the force of streaming lies in fermentation (Embden-Meyerhof-Parnas pathway) and not in respiration. That the force of streaming increases through suppression of respiration can be understood as a result of the acceleration of fermentation through the Pasteur effect (79).

Although there should be no chemical difference between the ATP that can be obtained with high efficiency by respiration and the ATP that is produced with lower efficiency by the process of fermentation, a striking difference between the two is thus observed in their action on one physiological activity, that of the generation of the force of streaming. This problem will be considered in the following section.

Intraplasmodial location of ATP synthesis and its significance in motive force production.—It was ascertained that in the slime mold *Physarum polycephalum* there are cytochromes *a,b,c* (79) and cytochrome oxidase (51, 79, 80); and that succinic dehydrogenase is concentrated in mitochondria (51). The presence of mitochondria and details of their morphology have been confirmed through electron microscopy by Terada (81). That respiration is centered in the mitochondria seems to be no exception with the slime mold. If so, we can assume that the ATP synthesized by respiration is produced in the mitochondria, and that the ATP synthesized through the glycolytic process is produced in the so-called soluble part, that is, the ground cytoplasm. That ATP is produced in different places depending upon whether it is synthesized through respiration or glycolysis means that even chemically identical ATP can serve different physiological functions with respect to living protoplasm.

The site of the mechanochemical system, that is, the site where the motive force is generated, is not the granules of the cytoplasm but the ground cytoplasm. If myosin-B of the slime mold, under the effect of ATP, causes changes in the form or organization pattern of molecules and if these changes have a direct bearing on the generation of the motive force, ATP should at least be present in a place where it can react on this protein if it is to be used for movement. We can hence infer that it is because of such a difference in the location of ATP production that fermentation energy is readily available for the generation of the motive force while respiration energy is not.

There are many cases in which respiration energy is used for protoplasmic streaming in plants (82, 83, 84). A prerequisite condition in these

cases is that the ATP of the mitochondria is released with relative ease and dispersed in the cytoplasm. Even if the average level of high-energy phosphate in the cell as a whole rises through respiration, it has little utility for protoplasmic movement if that high-energy phosphate hardly exudes in the ground plasm. Hatano & Takeuchi (71) have measured quantitatively the entire ATP content of a plasmodium treated either with low oxygen tension, 2,4-dinitrophenol, or monoiodoacetate. In these cases they did not necessarily observe a parallelism between the average ATP level of the entire plasmodium and the motive force of the streaming. Needless to say, what represents the direct energy source of protoplasmic movement is not the ATP level of the protoplasmic system as a whole, but the ATP level at the locus where the motive force is generated. Therefore it is rather natural that there is no clear parallelism between the ATP level of the plasmodium as a whole and the force of streaming. But it has been experimentally demonstrated, as stated above, that if the ATP level of the ground plasm is artificially raised through microinjection, the movement becomes more lively up to a certain limit. This shows that the ATP level of the ground plasm of the slime mold is in a suboptimal state for streaming, under normal conditions.

In summary, since glycolysis as well as generation of the force of protoplasmic streaming occurs in the ground plasm, the energy obtained through glycolysis can be easily used for movement, while the energy obtained through respiration gains significance as the energy source for movement only when it is released readily from the mitochondria and can come in contact with the mechanochemical system of contractile proteins.

Since there is lively respiration in the slime mold when it grows actively, it may be assumed that the ATP from the mitochondria is consumed for the synthesis of protein, nucleic acids, etc. Each of the various physiological activities which are going on simultaneously in the living protoplasm needs its own energy and it is an important problem for physiologists to determine through what mechanism this energy allotment is being coordinated and controlled. In the slime mold, as there is a great difference in the utility of ATP obtained from respiration and glycolysis as stated above, the balance between respiration and glycolysis must be closely related with the supply of energy for protoplasmic streaming and must be connected with the coordination of movement as well.

Even though chemically identical, ATP molecules in the living system cannot be equivalent from the standpoint of ATP utilization if the sites of their production and their modes of existence within the cell are different. This conclusion must also be true of other physiological phenomena besides movement. As far as the author is aware, the difference in the utility of ATP from different sites within the living cell in relation to physiological activity has not been emphasized up to the present and no concrete examples have been discussed, but it is an important problem in the physiology of the living organism.

LITERATURE CITED

1. Kamiya, N., *Protoplasmic Streaming* (*Protoplasmatologia*, **VIII/3/a**, Springer-Verlag, Wien, Austria, 199 pp., 1959)
2. Kamiya, N., and Kuroda, K., *Botan. Mag.* (*Tokyo*), **69**, 544–54 (1956)
3. Hayashi, T., *Botan. Mag.* (*Tokyo*), **65**, 51–55 (1952)
4. Nichols, S. P., *Bull. Torrey Botan. Club*, **52**, 351–63 (1925)
5. Linsbauer, K., *Protoplasma*, **5**, 563–621 (1929)
6. Peterfi, T., and Yamaha, G., *Protoplasma*, **12**, 279–86 (1931)
7. Breckheimer-Beyrich, H., *Ber. deut. botan. Ges.*, **67**, 86–92 (1954)
8. Jarosch, R., *Osterr. Akad. Wiss. Math.-naturw. Kl., Anz.*, **6**, 58–60 (1956)
9. Jarosch, R., *Phyton* (*Buenos Aires*), **6**, 87–107 (1956)
10. Jarosch, R., *Biochim. et Biophys. Acta.*, **25**, 204–5 (1957)
11. Jarosch, R., *Protoplasma*, **50**, 93–108 (1958)
12. Frey-Wyssling, A., *Exp. Cell. Research*, **1**, Suppl., 33–42 (1947/49)
13. Frey-Wyssling, A., *Submicroscopic Morphology of Protoplasma* (Elsevier Publ. Co., Amsterdam, Holland and New York, N.Y., 1953)
14. Frey-Wyssling, A., *Die submikroskopische Struktur des Cytoplasmas*, (*Protoplasmatologia*, **II/A/2**, Springer-Verlag, Vienna, Austria, 244 pp., 1955)
15. Loewy, A. G., *Proc. Am. Phil. Soc.*, **93**, 326–29 (1949)
16. Kishimoto, U., *J. Gen. Physiol.*, **41**, 1223–44 (1958)
17. Kamiya, N., and Kuroda, K., *Proc. Japan Acad.*, **33**, 149–52 (1957)
18. Kamiya, N., *Protoplasmic streaming* (9th Inter. Botan. Congr., Montreal, Canada, August 1959)
19. Kamiya, N., and Kuroda, K., *Proc. Japan Acad.*, **33**, 201–5 (1957)
20. Fetzmann, E. L., *Protoplasma*, **49**, 549–56 (1958)
21. Valkanov, A., *Protoplasma*, **20**, 20–30 (1934)
22. Kamiya, N., and Kuroda, K., *Protoplasma*, **50**, 144–48 (1958)
23. Hayashi, T., *Botan. Mag.* (*Tokyo*), **70**, 168–74 (1957)
24. Kamiya, N., and Kuroda, K., *Proc. Japan Acad.*, **33**, 403–6 (1957)
25. Hatano, S., and Nakajima, H., *ATP Content and ATP-dephosphorylating Activity of Nitella* (24th Ann. Meeting Botan. Soc. Japan, Sendai, September 1959)
26. Cohn, W. E., and Carter, C. E., *J. Am. Chem. Soc.*, **72**, 4273–75 (1950)
27. Takata, M., *Kagaku* (*Tokyo*), **28**, 142 (1958)
28. Sandan, T., *Botan. Mag.* (*Tokyo*), **72**, 337–41 (1959)
29. Abe, S., *Kagaku* (*Tokyo*), **29**, 361–62 (1959)
30. Kishimoto, U., and Akabori, H., *J. Gen. Physiol.*, **42**, 1167–83 (1959)
31. Smirnova, N. A., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **2**, 578–80 (1955)
32. Hanson, T., and Huxley, H. E., *Symposia Soc. Exptl. Biol.*, **9**, 288–64 (1955)
33. Huxley, H. E., *Progr. in Biophys.*, **7**, 255–318 (1957)
34. Weber, H. H., *The Motility of Muscle and Cells* (Harvard Univ. Press, Cambridge, Mass., 69 pp., 1958)
35. Jahn, T. L., and Rinaldi, R. A., *Biol. Bull.* (In press)
36. Jarosch, R., *Protoplasma*, **50**, 277–89 (1959)
37. Kamiya, N., *Protoplasma*, **45**, 513–24 (1956)
38. Camp, W. G., *Bull. Torrey Botan. Club*, **63**, 205–10 (1936)
39. Howard, F. L., *Am. J. Botany*, **18**, 116–33 (1931)
40. Jahn, A., *Z. Botan.*, **27**, 193–250 (1934)
41. Mangenot, G., *Rev. cytol. et biol. vegetables*, **1**, 20–66 (1934)

42. Camp, W. G., *Bull. Torrey Botan. Club.*, **64**, 307–35 (1937)
43. Cohen, A. L., *Botan. Gaz.*, **101**, 243–75 (1939)
44. Cohen, A. L., *Botan. Gaz.*, **103**, 205–24 (1941)
45. Dalleux, G., *Rev. cytol. et biol. vegetales*, **4**, 123–82 (1940)
46. Martin, G. W., *Botan. Rev.*, **6**, 356–88 (1940)
47. Lewis, W. H., in *The Structure of Protoplasm*, 163–97 (Seifriz, W., Ed., Monograph Am. Soc. Plant Physiol., Ames, Iowa, 1942)
48. Seifriz, W., in *The Structure of Protoplasm*, 245–64 (Seifriz, W., Ed., Monograph Am. Soc. Plant Physiol., Ames, Iowa, 1942)
49. Sobels, J. C., *Nutrition de quelques myxomycetes en cultures pures et associees et leurs proprietes antibiotiques* (Doctoral thesis, University of Utrecht, Utrecht, The Netherlands, 1950)
50. Andresen, N., and Pollock, B. M., *Compt. rend. tran. lab. Carlsberg, Ser. chim.*, **28**, 247–64 (1952)
51. Johnson, G. T., and Moos, C., *J. Cellular Comp. Physiol.*, **48**, 243–52 (1956)
52. Kamiya, N., *Protein, Nucleic Acid, Enzyme (Tokyo)*, **4**, 177–85 (1959)
53. Kamiya, N., *Cytologia (Tokyo)*, **15**, 183–93 (1950)
54. Kamiya, N., and Kuroda, K., *Protoplasma*, **49**, 1–4 (1958)
55. Kamiya, N., *Cytologia (Tokyo)*, **15**, 194–204 (1950)
56. Kamiya, N., *Protoplasma*, **39**, 344–57 (1950 c)
57. Kamiya, N., *Science*, **92**, 462–63 (1940)
58. Kamiya, N., in *The Structure of Protoplasm*, 199–244 (Seifriz, W., Ed., Monograph Am. Soc. Plant Physiol., Ames, Iowa, 1942)
59. Kamiya, N., *Ann. Rept. Sci. Works, Fac. Sci., Oska Univ.*, **1**, 53–83 (1953)
60. Kamiya, N., Abe, S., and Nakajima, H., *Proc. Japan Acad.*, **33**, 206–10 (1957)
61. Nakajima, H., *A Contractile Protein in the Myxomycete Plasmodium* (22nd Ann. Meet. Botan. Soc. Japan, Tokyo, Japan, October 1957)
62. Nakajima, H., *Symposia on Enzyme Chem. (Japan)*, **14** (In press)
63. Loewy, A. G., *J. Cellular Comp. Physiol.*, **40**, 127–56 (1952)
64. Ts'o, P. O. P., Bonner, J., Eggman, L., and Vinograd, J., *J. Gen. Physiol.*, **39**, 325–47 (1956)
65. Ts'o, P. O. P., Eggman, L., and Vinograd, J., *J. Gen. Physiol.*, **39**, 801–12 (1956)
66. Ts'o, P. O. P., Eggman, L., and Vinograd, J., *Archiv. Biochem. Biophys.*, **66**, 64–70 (1957)
67. Ts'o, P. O. P., Eggman, L., and Vinograd, J., *Biochim. et Biophys. Acta*, **25**, 532–42 (1957)
68. Nakajima, H., *Seitai no Kagaku (Medical Science)*, **7**, 256–59 (1956)
69. Kamiya, N., Nakajima, H., and Abe, S., *Protoplasma*, **48**, 94–112 (1957)
70. Takata, M., *Physiological Studies on the Protoplasmic Streaming in Plasmodia by Means of Microdissection.* (22nd Ann. Meet. Botan. Soc. Japan, Tokyo, Japan, October (1957)
71. Hatano, S., and Takeuchi, I., *Protoplasma*, (In press)
72. Allen, P. J. and Price, W. H., *Amer. J. Botany*, **37**, 393–402 (1950)
73. Loewy, A. G., *J. Cellular Comp. Physiol.*, **35**, 151–53 (1950)
74. Kamiya, N., Abe, S., and Nakajima, H., *Proc. Japan Acad.*, **33**, 407–9 (1957)
75. Kamiya, N., Abe, S., and Nakajima, H., *Proc. Japan Acad.*, **34**, 530–33 (1953)
76. Ohta, J., *Cytologia (Tokyo)* **17**, 210–18 (1952 a)
77. Ohta, J., *Cytologia (Tokyo)* **17**, 300–10 (1952 b)

78. Abe, S., Nakajima, H., and Kamiya, N., *Proc. Japan Acad.*, **34**, 697–99 (1958)
79. Ohta, J., *J. Biochem., (Tokyo)* **41**, 489–97 (1954)
80. Ward, J. M., *Science*, **127**, 596 (1957)
81. Terada, T., *Electronmicroscopic Studies on the Fine Structure of Myxomycete Plasmodia, III* (23rd Ann. Meet. Botan. Soc. Japan Fukuoka, Japan, October 1958)
82. Bottelier, H., *Rec. trav. botan. neerl.*, **32**, 287–92 (1935)
83. Sweeney, B. M., and Thimann, K. V., *J. Gen. Physiol.*, **21**, 439–61 (1938)
84. Kelso, J. M., and Turner, J. S., *Australian J. Biol. Sci.*, **8**, 19–35 (1955)

PRODUCTIVITY OF THE OCEANS[1]

By E. Steemann Nielsen
Royal Danish School of Pharmacy, Copenhagen, Denmark

Introduction

The primary production of the oceans is due almost exclusively to the photoautotrophic phytoplankton, a fact recognized early by oceanographers. The investigation of quantitative aspects of oceanic plankton started at the end of the last century. Hensen, the leader of the German "Plankton Expedition" in the North Atlantic in 1899 (58), must be mentioned first. No techniques for estimating oceanic productivity were available at that time, but instead an attempt was made to measure the quantity of phytoplankton below a unit of the sea surface. The standing stock of phytoplankton may be presented as volume or wet weight (equalling the biomass) which may be given as organic matter or carbon by use of appropriate conversion factors.

The word production has sometimes been used synonymously with standing stock. Although in nature there ordinarily appears to be a rather high correlation between the standing stock of phytoplankton and the primary production, it is nevertheless important to distinguish sharply between these two.

Investigations of organic productivity in the sea were first started in the twenties of this century, the pioneers being Atkins (2, 3) and Gaarder & Gran (42). At present, the study of phytoplankton production is one of the central problems of marine science. Productivity has been discussed in numerous recent marine-biological and physico-oceanographical articles, and it would be quite impossible to mention them all in a review of the present kind. The readers are referred to the contributions to the recent Plankton Symposium, 1957, *Measurements of Primary Production in the Sea* (*Rappt. et Procès-Verb. des Reunions,* **144,** 1958). Even in that volume, the literature is not listed exhaustively.

The Phytoplankton Standing Stock

Methods.—It seems very appropriate in a review of oceanic productivity to include at least a short account of the main literature concerning the standing stock of phytoplankton. Some of the most outstanding facts concerning productivity have been obtained by studying the size and the variation of the standing stock.

To measure the standing stock of the phytoplankton, the organisms suspended in the water must first be concentrated. This is possible by filtration, centrifugation, or sedimentation; subsequently either the number of plankton organisms may be counted or the organic matter estimated by some chemical means. If counting is used it is necessary to measure the volume

[1] The survey of literature pertaining to this review was concluded August 1, 1959.

of the different species found and to use a conversion factor (volume/organic matter). Braarud (15) has presented a very useful review of counting methods for the determination of the standing stock ("crop") of phytoplankton.

In filtration techniques different materials have been used: bolting silk, filter paper, fine-meshed metal sieves, sand, and membrane (colloidal) filters. Whereas bolting silk ordinarily has been used for making nets, the other materials can be used only for filtering relatively small water samples collected previously in the sea.

When quantitative methods were introduced at the end of the last century, nets of bolting silk were used exclusively. The finest silk at that time was No. 20, having a mesh diameter of about 60 μ. Silk No. 25, used more recently, has a diameter of about 50 μ. Unfortunately, the size of most marine phytoplankton organisms is less than these values. Vertically hauled nets such as those used by Hensen were thus inadequate for collecting the whole of the phytoplankton. This fact was shown by Lohmann (80), during the first years of this century, but the general effect on the contemporary plankton science was surprisingly little. It seems to have been tacitly assumed that although the main bulk of the algae is lost through the meshes of the nets, the larger retained species are the main producers of organic matter; or that the part of the phytoplankton retained by the net is a rather consistent fraction of the total phytoplankton. These assumptions have not been shown to be correct (54, 103, 127, 140, 160).

The techniques using silk nets for quantitatively measuring phytoplankton must now be considered as of historical interest only. The participants in the *International Symposium on Measurements of Primary Production in the Sea* in 1957 at Bergen unanimously condemned further application of net methods in quantitative studies of phytoplankton. The only filtration method that may presently be recommended to some extent employs membrane filters. After terminating the filtration, these filters are made transparent with oil, for example (45). Regrettably, small naked forms are often more or less destroyed.

Centrifugation in marine work was introduced at the beginning of this century by Lohmann (80). The plankton algae in a certain volume of water are concentrated in a small drop and counted under a microscope; however, the losses may be rather large (111, 124). The technique has some advantages, nevertheless, because living algae may be investigated. Lohmann (81) and Hentschel (60), using this technique, have contributed much to the understanding of the production of the ocean and coastal waters.

Several variations of sedimentation methods were introduced by Utermöhl (148). One of these is combined with a reversed microscope and is used presently by most marine workers engaged in quantitative phytoplankton science. Unfortunately it is necessary to preserve the algae, a process that destroys several small naked flagellates which sometimes constitute a major bulk of the biomass (50).

Lohmann (80) first introduced a method allowing computation of the

biomass and the content of organic matter from plankton counts. He even considered the complication due to inclusion of the cell sap in the volume of the cells. Cushing (28) recently reviewed the various conversion factors applied by different workers when computing content of organic matter from plankton counts.

It is not feasible to concentrate total phytoplankton from really large volumes of water. When estimating organic matter by chemical methods, extremely sensitive techniques must therefore be employed. Most of these are rather complicated and hence still only little used. Von Brand (17) presented methods for determining nitrogen and carbon in small amounts of plankton and Krey (77) described a method for the determination of protein. It is impossible, however, to distinguish between phytoplankton and organisms of other kinds, or even detritus. As inorganic phosphate is readily adsorbed by all particles, particulate phosphorus is often a rather poor indicator of the organic matter in the organisms.

The photosynthetic pigments are the only components simultaneously specific for all photoautotrophic plankton algae and relatively simple to determine. Either total pigment, total chlorphyll, or the single pigments may be measured. Pigment analyses were started about 1930 using net samples. Krey (75) introduced a technique for adequately filtering the sea water. Pigment analyses—primarily chlorophyll determinations—have been used with relative frequency in recent years (4, 5, 26, 33, 44, 46, 62, 65, 72, 78, 93, 95, 100, 101, 109, 110). A spectrophotometric technique was introduced by Richards & Thompson (91). Although encumbered with many pitfalls, pigment analyses have several advantages for estimating phytoplankton organic matter.

Some of the pitfalls are: (*a*) a considerable part of the chlorophyll found in a sea water sample may be a constituent of detritus (44), and (*b*) the extraction of chlorophyll from plankton algae may be incomplete unless the cell walls are mechanically destroyed (109, 114). Krey 1958 (79) reviewed the literature concerning the chemical methods of estimating phytoplankton biomass. He presents a survey of the chlorophyll content of phytoplankton showing the ratios of chlorophyll to both organic matter and cell volume (also 28). The range of variation is tenfold. It does not seem possible at present to recommend standard techniques for pigment analysis.

Some workers have used suspended matter as an index of the quantity of plankton. The suspended matter is either measured optically by means of the Tyndall effect, as done by Jerlov (67), by filtering and weighing (54, 76), or by simply measuring the transparency of the water (53). In the open ocean Hentschel (60) noticed the close agreement between the colour of the sea and the quantity of plankton; but suspended matter is not always a reliable index of coastal waters, where great quantities of detritus and inorganic particles may be found.

Results.—More or less disregarding the measurements of phytoplankton quantities using nets we may start with the outstanding work of Lohmann (80) done in the Western Baltic. Weekly centrifuge measurements of the

phytoplankton volume from the surface to a depth of 15 m. were made during a whole year. Although the absolute values are definitely too low, the general shape of the annual curve of volumes agrees well with modern investigations of organic productivity in these waters. During the "Deutschland" Expedition (1911), Lohmann made extensive investigations of the same kind in the tropical and subtropical parts of the North and South Atlantic but without computing the volumes from the cell counts. His general conclusions (81) about productivity in these water must still be considered valid.

The work was continued by Hentschel (59, 60) during the "Meteor" Expedition, 1925–1927, covering the whole of the South Atlantic and parts of the North Atlantic. The most amazing result—although already shown on smaller scale by Lohmann (81)—was the demonstration of the uniform quantitative distribution of the phytoplankton in the parts of the oceans where the same hydrographical conditions were found. Phytoplankton numbers could be treated just like properties such as salinity.

Between the two world wars quantitative phytoplankton investigations were made in many quarters throughout the world, unfortunately however, ordinarily using nets of fine silk. It would of course be wrong to maintain that this work was without any value. The several expeditions of the British "Discovery" thus added noteworthy contributions to the understanding of the phytoplankton population in the Antarctic (55). When the nets collect as much phytoplankton as they do generally in the Antarctic during the summer, a large stock of phytoplankton must be present. On the other hand, small catches by no means need to be, and very often are not, indicative of an inconsiderable stock of phytoplankton. This must also be remembered if preserved plankton and an otherwise adequate technique are employed (50). A considerable part of the producers may have been more or less completely destroyed.

The following references (8, 9, 13, 14, 47, 48, 49, 52, 125, 128) include some of the more important, mainly Scandinavian, contributions to quantitative phytoplankton investigation. They combined either a centrifuge or a sedimentation technique with counting of cells. These references (4, 62, 64, 65, 72, 73, 77, 84, 95, 100) present a series of important contributions in which a chemical determination of some kind was used to measure the standing stock of phytoplankton. It is impossible to present here a full account of the literature dealing with quantitative phytoplankton investigations. A fairly complete list may be found in *Current Bibliography* under the headings 3)c and 3)d in *Journal du Conseil,* Volumes 1 to 24. The investigation of the standing stock of phytoplankton has added much to the understanding of oceanic primary productivity.

The Production of Organic Matter

In the open ocean the plankton algae are distributed throughout the whole photic layer which is ordinarily about 50 to 125 m. deep. The production of organic matter takes place in the same layer. Although the rate

of production per surface unit is ordinarily not very low by comparison with the rate found in several terrestrial vegetations, the measurements are difficult just because of the considerable vertical extension of the photic layer. Thus, the amount of algae found in a single litre of water from the photic zone of the ocean equals that found below a surface area of about 0.1 cm^2. If the rate of production per square m. per day is put at about 200 mg.C—a relatively high rate in the open ocean—the average production per litre per day in the photic zone is about 2 μg. C (equivalent to 5 μg. O_2). This means that on the average only 0.01 per cent of the total carbon dioxide found in the water is assimilated daily or that 0.1 per cent of the oxygen found is produced. If the rate of production were to be measured either by carbon uptake or oxygen production using ordinary chemical methods, an experiment of rather long duration would be necessary. The thinner the photic layer and the higher the rate of production per surface unit the shorter may be the duration of the experiments. In productive coastal areas where the depth of the photic layer is shallow it is possible to measure productivity in experiments lasting only a day.

Methods.—The first investigation of actual organic production by phytoplankton in the sea was made in 1922 by Atkins (2) in the English Channel. He proceeded by determining the loss of carbon dioxide under 1 sq.m. of surface from the end of winter to the height of summer. As the loss in carbon dioxide equals the amount used in photosynthesis, a minimum value for the organic production in six months could be calculated. For example, it was not possible to consider the exchange of CO_2 with the atmosphere or the regeneration of CO_2 because of the respiration of all organisms including the plankton algae. Atkins (3) later used the loss of phosphate under 1 sq.m. of surface in the same way and Cooper (23) based his estimate—also for the English Channel—on the change in the content of oxygen and nitrate.

The different methods have been used relatively little (12, 20, 21, 55, 70, 74, 152) because of many difficulties (24). Steele (121, 122), however, has found the northern part of the North Sea rather well suited for the method. He used the loss of phosphate in the photic layer during the production season and corrected for phosphate regeneration by assuming the regeneration rate to be the same in the photic layer and the water layers below, where no uptake of phosphate takes place.

Estimates of the undersaturation of oxygen in a layer below the photosynthetic zone in the southwestern part of the North Atlantic were used by Seiwell (113) as a measure of the production of matter by plankton algae. He assumed that the annual amount of organic matter produced in the photic zone was finally oxidized in the layer mentioned above. According to Redfield (90), however, the oxygen deficiency in the water layer arose at a time when the water masses forming this layer were nearer the surface much farther north. Seiwell's estimates are thus hardly correct. The same must be true of similar estimates (99, 100).

In Russia (39, 157) the daily variation in the oxygen content of the

water between morning and afternoon has been used as a means of estimating organic production. It is a matter of course that this method can be employed only in very productive areas.

An experimental method for measuring organic production in the sea was introduced 30 years ago by Gaarder & Gran (42) and has since been used with slight modifications (136). In its most recommended form, water samples are collected from various depths and siphoned into bottles with glass stoppers. Some of the bottles are used for determining the concentration of oxygen before the start of the experiment, using a Winkler technique. The other bottles are again lowered to the depths from where the samples came and kept there fixed to a line hanging down from an anchored buoy for 24 hr. As some of the bottles are wrapped in black material, it is possible to determine the amount of both respiration and photosynthesis going on in the water samples. The oxygen content in the black bottles minus that in the initial bottle represents the rate of respiration by all of the organisms present, whereas the oxygen content in the black bottles minus that in the clear bottles represents the rate of photosynthesis by the phytoplankton.

The lower limit of this "oxygen technique" depends on the sensitivity of the Winkler method. In a single titration it is impossible to determine oxygen to better than 0.02 mg.O_2/l. The precision is generally less in coastal water. Nevertheless, in productive coastal water the oxygen technique for measuring organic productivity is ordinarily applicable (97, 123, 126, 129) but many pitfalls may turn up (136). In clear oceanic water the oxygen technique is of little use for measuring organic productivity. The rate of photosynthesis is much too low to allow experiments of short duration. Severe complications arise in experiments of longer duration due to the development of a heavy bacterial growth when sea water is enclosed in bottles (161). The bacterial growth seems to start earlier when the concentration of phytoplankton is very low (41, 136). Light, in some way, is able to reduce the bacterial activity in the bottles containing oligotrophic ocean water (130, 131, 133; see also 149). The difference in oxygen content between light and dark bottles after an experiment of long duration with oligotrophic water has scarcely anything to do with photosynthesis, as some workers have assumed (92, 93, 96, 108).

A technique for measuring organic productivity in the oceans based on radioactive tracer ^{14}C was used for the first time during the Danish "Galathea" Expedition 1950–52. The technique was described by Steemann Nielsen (130). Further details and modifications are described (1, 7, 19, 37, 68, 103, 118, 136, 140).

Incorporation of the tracer in the organic matter of the plankton algae is used as a measure of the production. A solution with a definite amount of $NaH^{14}CO_3$ in sealed ampoules is added to the sea water before an experiment. The content of ^{14}C in the plankton after the experiment is multiplied by a factor corresponding to the ratio between CO_2 (total) and $^{14}CO_2$ in the

water at the beginning of the experiment. The amount of ^{14}C assimilated is determined by measuring the β-radiation from the plankton retained by a collodion filter.

Some minor corrections have to be introduced. Experiments have shown (132) that in light about 50 to 70 per cent of the CO_2 produced by respiration is fixed again by photosynthesis before its final release. Since at high light intensities the rate of respiration in phytoplankton collected in nature is about 10 per cent of the rate of light-saturated photosynthesis (141) we have to add 6 per cent to the actual measurements at high light intensities in order to present gross photosynthesis. This concerns only experiments of relatively short duration.

In ^{14}C experiments of very long duration where practically all of the organic matter to be used in respiration is produced during the experiments, all CO_2 respired will be of the same kind as that assimilated. Net production is thus measured in such experiments. Because other workers (108) used experiments of very long duration, it is easy to explain the controversy with the present writer (who used only short experimental times) as to what is really measured by the ^{14}C technique. Experiments of long duration with oceanic plankton (but not always with coastal plankton) are to be avoided because the real oceanic algae are often quite unable to stand the conditions in experimental bottles for such long periods (60). In experiments without stirring, where the algae may be concentrated by sedimentation, short periods are essential.

Another correction for dark fixation of CO_2 by the plankton algae and the other organisms must be made. It is advisable to make simultaneous experiments in dark bottles. In areas where the population of heterotrophic organisms is high compared with those of autotrophic algae it is mandatory to make such dark experiments and to subtract the dark fixation from the light fixation in order to estimate the rate of photosynthesis. As the rate of dark fixation in ordinary sea water generally is only about 1 to 3 per cent of that in saturated light, it is usually permissible to correct arbitrarily for dark fixation.

For the determination of the production of matter under 1 sq.m. of surface, three different modifications of the method may be employed. The best modification is the *"in situ"* method resembling Gaarder and Gran's oxygen method (42). Instead of measuring oxygen metabolism in the bottles suspended at different depths the intensity of photosynthesis is determined by the ^{14}C technique. To estimate the total photosynthesis below 1 sq.m. of surface of the sea the determinations at each of the depths are presented graphically, see Figure 1. The area to the left of the curve represents the photosynthesis below the surface. This method has been employed by many workers (18, 26, 50, 103, 118, 119, 120, 121, 122, 130, 137, 140, 154). It is not always applicable on a larger scale for expeditions on big ships as it is rather time consuming. The present writer prefers to make experiments during only a half day, either from sunrise to noon or from noon to sunset.

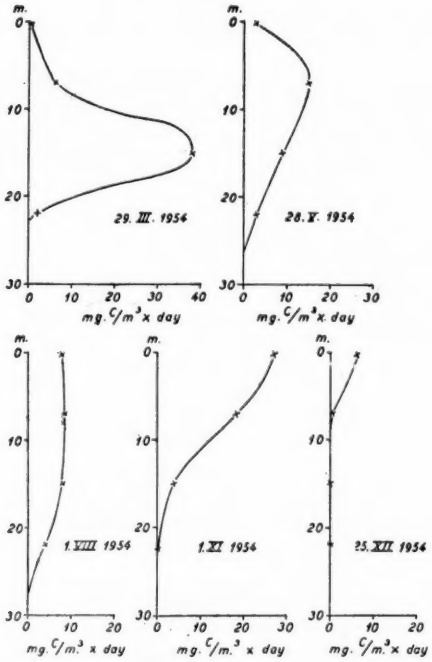


FIG. 1. Normal *in situ* experiments from all seasons in the Kattegat. The light conditions were favourable in all cases. The ordinate is depth in meters and the abscissa the mg. C per m.³ per day.

Rodhe (103) working in fresh water uses an experimental time of 24 hr.

In order to avoid having a big ship stationary for a relatively long period of the day, other modifications of the ^{14}C technique have been developed. Thus, the water samples collected from the different depths are illuminated on board the ship, either in a tank employing artificial light or by the natural illumination falling on the deck of the ship. Steemann Nielsen (130, 137) fixed the bottles containing the water samples to a rotating wheel and exposed them to a definite light intensity in a water-bath at the same temperature as that of the sea. From the value of the photosynthetic rate found at a given light intensity it is possible to calculate the production of matter throughout a period of 24 hr. We must first know the dependence of photosynthesis on the light intensity and, second, the light intensity variations during the day at the depths from which the samples originate.

In practice, however, such a procedure would be too time consuming.

Formulas have therefore been deduced for calculating the primary production by means of tank experiments. Whereas Steemann Nielsen (130, 136, 140) and Sorokin (118) tackled the problem empirically by simultaneously making experiments *in situ* and in the tank, Ryther (107) and Rodhe, *et al.* (104) deduced their formula more or less theoretically. The latter showed that their formula agrees with the empirical relationship used by Steemann Nielsen. The tank method has been used with relative frequency during recent years. Besides the references already quoted, the following may be listed (1, 7, 25, 29, 34, 35, 37, 51, 64, 68, 69, 71, 85, 86, 87, 110, 118, 119).

In the simulated *in situ* method, we finally have a procedure resembling the *in situ* method but practical when the ship is moving. Riley (93) introduced this method in a very simple form by suspending bottles with surface water in a tub on deck and determining the oxygen production. Berge (7) and Steemann Nielsen (136), using the ^{14}C technique, have taken samples from several depths of the photic layer. In order to imitate the light conditions under the surface, plates of neutral glasses with suitable light absorption characteristics are placed above the bottles with subsurface water. Berge (7) showed that such simulated *in situ* measurements did not significantly differ from those made on corresponding samples suspended simultaneously at the normal depths in the sea. UNESCO has provided funds for establishing an international center both for producing ampoules containing water with a definite amount of $NaH^{14}CO_3$ and for measuring the filters with the radioactive plankton. The center was opened in 1958 at Charlottenlund Castle, Denmark.

Gessner (43) and Ryther & Yentsch (109) have described methods for estimating the phytoplankton production from chlorophyll and light data. However, the rate of light-saturated photosynthesis at the same concentration of chlorophyll varies within wide limits (142). Only plankton from similar habitats at the same time of the year yield similar curves. It is thus advisable to use chlorophyll analyses for estimating the phytoplankton production only if we restrict ourselves to a definite habitat and a definite season; or if we introduce varying factors according to latitude, season, and depth for the relationship between the chlorophyll concentration and the rate of light-saturated photosynthesis. Unfortunately, even then, because of the difficulties in estimating chlorophyll, the results may often be relatively poor.

The cell-size decrease method of Cushing (27) must also be mentioned. By means of the decrease in the size of the cells, the number of divisions can be estimated between two observations in the sea. The method is applicable, however, only to diatoms and the diatom population must be identified as identical for both observations. This makes a general application of the method rather unlikely.

Gross and net production.—To evaluate the primary production in a

natural habitat it is necessary to know the size of both the gross production and the net production. Therefore, the rate of respiration must also be known. Gross production minus respiration equals net production.

In experiments with the usual dark and light bottle oxygen method, under conditions where this method is applicable, it is possible to measure the rate of the gross production by the autotrophic phytoplankton and the rate of respiration in all of the organisms present in the water sample. Instead of net primary production we measure net community production. Besides bacteria, many other heterotrophic plants may be found in a water sample. Finally, zooplankton organisms may contribute considerably to the total respiration. The rate of net primary production is always higher than the rate of net community production and in polluted waters it is much higher.

Steemann Nielsen & Hansen (141; see also 136, 140), have described a method for measuring exclusively the respiratory rate of an autotrophic phytoplankton population by means of the ^{14}C technique. A curve showing the rate of net photosynthesis as a function of light intensity is obtained; by extrapolation, a rather precise rate of respiration can be deduced. The method has been regularly used for several years during the cruises of the Danish research vessels. More than 100 such measurements have now been made. The rate of respiration (as a percentage of light-saturated photosynthesis) has been shown without exception to be low; about 10 per cent or less in phytoplankton collected in nature. This is true both for eutrophic and oligotrophic habitats.

Ryther (105) originally suggested that in unproductive waters the rate of respiration may be nearly as high as the rate of photosynthesis. This claim has been used to question the general validity of the ^{14}C technique (106, 110). The demonstration of the consistently low rate of respiration is thus important. It is of little importance that the technique measures something between net production and gross production. The difference between these two quantities at high light intensities is small.

Measurements.—Published estimates of the rate of primary production must be arranged according to both the method employed and the area investigated. Productive coastal regions must be mentioned first. The different methods and techniques employed have generally given about the same results. In Table I the published estimates of primary production in coastal areas covering at least half a year have been collected. The method employed is stated. Besides the estimates from coastal areas presented in Table I covering more or less a whole year, several other measurements of primary production have been published covering only shorter periods (18, 25, 29, 35, 64, 118, 140, 154).

Areas where the annual production is somewhat higher than the highest value presented in Table I are very likely to be found. It is not unreasonable to infer that a shallow eutrophic locality in the tropics perhaps may

TABLE I

ESTIMATES OF RATES OF PRIMARY PRODUCTION
(Gross Production, If Not Otherwise Mentioned)

Area		References	Method	Period	Rate of Production
					(gm. C/m².)
English Channel		2, 3, 22, 23	Decrease in phosphate	Half Year	
			Decrease in CO_2	Half Year	39–101 (net)
			Decrease in nitrate	Half Year	
Northern North Sea		121, 122	Decrease in phosphate	Year	54–127 (net)
Danish Waters	The Sound	126	Oxygen (experimental)	Year	39
	Kattegat	137	Carbon-14	Year	61
	Great Belt	137	Carbon-14	Year	60
	Isefjord	129	Oxygen (experimental)	Year	175
	Limfjord	50, 137	Carbon-14	Year	105
	Dybsøfjord	50, 137	Carbon-14	Year	6
Coast of West Greenland	Outside Narssak	137	Carbon-14	Year	29
	Mouth of Godthåb Fjord	137	Carbon-14	Year	98
	Inner part of Godthåb Fjord	137	Carbon-14	Year	95
Barents Sea		74	Decrease in phosphate	Year	92 (net)
U.S.A. Atlantic Coast	Long Island Sound	94, 110	Oxygen (experimental)	Year	380
	Georges Bank	95	Oxygen (experimental)	Year	80–90 (net)
	Continental Shelf off New York				
	(a) 25–50 m. depth	110	Chlorophyll	Year	160
	(b) 50–1000 m. depth	110	Chlorophyll	Year	135
	(c) 1000–2000 m. depth	110	Chlorophyll	Year	100

produce up to about 1 kg. C/m.^{2} per year. The even higher rates of production, such as shown in outdoor mass cultures of green algae, (145) are not very likely to be found in nature. Such high rates are possible only if carbon dioxide is continuously bubbled through the medium—a condition not found in marine habitats.

Estimates of the rate of primary production in the open ocean are much fewer than those from coastal areas. In lower latitudes without sharp seasonal variations it is impossible to use the consumption of phosphate, nitrate, or the like in estimating the rate of primary production. Similarly, it seems hardly realistic to use properties such as the degree of undersaturation of oxygen below the photic zone or similar properties for estimating the rate of primary production. Measurements with the ordinary dark and light bottle oxygen technique (10, 93) in oligotrophic water have given erroneous results because of the extensive development of bacteria in the experimental bottles (130, 136). Reliable measurements of oceanic primary production at lower latitudes have been obtained using the ^{14}C technique. An estimation based on measurements of the standing stock of algae, for example by chlorophyll measurements, also gives useful information (26, 62, 63, 64, 72, 109).

Measurements of organic production in an oceanic area made once, or only during a part of the year, are relatively numerous both in the Atlantic and in the Pacific (7, 26, 35, 62, 64, 72, 118, 120, 137, 140). Thus, during a month, September to October, Currie (26) made seven series of *in situ* measurements at a fixed station in the North Atlantic (40°N, 20°W). The ^{14}C technique was used. In the Norwegian Sea, Berge in 1958 (7) worked a close net of productivity stations during a single summer month. It was shown that the rate of production, both per volume at a definite light intensity and per surface unit with standard light conditions, may be treated just as chemical or physical properties like salinity and temperature. Investigations in other oceanic areas, although made on a somewhat smaller scale, have given the same picture.

A common feature for most tropical and subtropical parts of the oceans is the low range of variation in the values obtained within hydrographically uniform areas. This is true both for the plankton counts (60, 81) and for the measurements of organic production by the ^{14}C technique (62, 72, 140). During the Meteor Expedition (60) it was shown that ordinarily the seasonal variations were negligible in these waters. It is thus possible to use single measurements of organic production as representative of the mean annual daily production rate. By combining Hentschel's "Meteor" data on the standing stock in the South Atlantic and the "Galathea" measurements of organic production in the same ocean it was possible to develop a chart, Figure 2, showing the distribution of the annual net production (140).

According to this chart, three regions of about equal area are found in the South Atlantic, one with an annual rate below 30 gm. C, one with a rate between 30 and 60 gm. C and one with a rate between 60 and 100 gm. C.

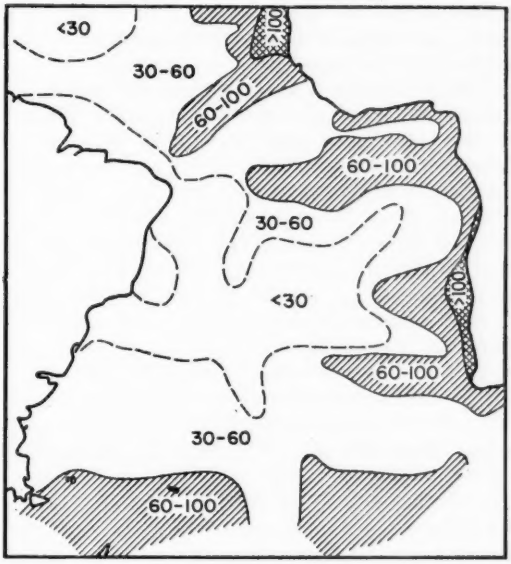


FIG. 2. Distribution of annual net production in the South Atlantic Ocean (gm. C per sq. m.).

Finally, some smaller regions, with upwelling, are found with rates above 100 gm. C per year. For antarctic waters the values given were consistent with the values published by Hart (55) based on the reduction in phosphate in the photic zone during the production season. Bogoyavlenskii (12) has published estimates of organic production in the northern part of the North Pacific based on measurements of phosphate reduction during the production season. They are similar to those from corresponding parts of the South Atlantic.

The most barren areas of the oceans are those with typically "old" surface water in the photosynthetic zone. This water has been transported from a distant region and the long lapse of time since the water entered the photosynthetic layer has resulted in a decrease in the nutrient content. The centres of the midoceanic anticyclonic eddies such as the Sargasso Sea are typical areas of that kind.

By contrast, "new" surface water in the photosynthetic layer is found in the upwelling areas along the western coasts of the continents, where the highest rates of oceanic production is found. Areas with relatively "new" surface water are found in the divergences at both sides of the equatorial countercurrents in the different oceans. The organic production

near the equator is thus normally relatively high, in sharp contrast to the conditions in the midoceanic areas at about latitudes 15° to 30° both in the northern and the southern hemispheres.

Total organic production in the oceans.—Rabinowitch (89) estimated the average annual organic production for all seas to be 375 gm. C per sq. m. The estimate was based on the assumption, put forward by Riley (93), that no great difference should exist between the annual production in the deep blue tropical parts of the oceans and in the coastal waters of temperate latitudes. This idea was in sharp contrast to estimates of the standing crop such as those made during the German Expeditions with the "Deutschland" (81) and the "Meteor" (60). Steemann Nielsen (130, 131, 140), therefore, rejected the former estimate on the grounds that the oxygen-technique used in oligotrophic waters is unreliable because of the development of a considerable growth of bacteria.

An estimate for oceanic regions was hence made by using only the results obtained by the ^{14}C technique. These results were in complete agreement with the German measurements of the standing stock. They were also in complete agreement with the purely theoretical approach by Sverdrup (144). According to Steemann Nielsen (140) the average annual gross production for all seas is about 55 to 70 gm. C/m^2. If we assume a 40 per cent loss through respiration and a sea area of 361×10^6 km^2., the total net production per year for all seas is about 1.2 to 1.5×10^{10} tons carbon. This figure is practically the same as that estimated by Schroeder (112) for the production on land but only one-tenth of Rabinowitch's estimate of the production in the sea.

The Factors Governing Organic Production

The different factors influencing the rate of primary production in the sea, such as the replenishment of nutrients, light, grazing, etc., cannot be traced separately. They are generally involved in such a way that all of them appear to act as limiting factors at the same time.

Two German scientists, Brandt and Nathansohn, were pioneers in exploring the background of primary production in the sea. A survey of the literature can be found (153). At the turn of the century Brandt (16a) suggested that phytoplankton production must depend upon the supplies of nitrate and phosphate in the sea but some of his ideas about the metabolism of the nutrient salts were obviously incorrect. His views must be supplemented by those of his great antagonist Nathansohn (88), who was the first to emphasize the importance of dynamic causes for the productivity in the sea, stressing the importance of grazing by the zooplankton as later did Harvey, *et al.* (57). On the other hand, Nathansohn denied the influence of deficiencies in nutrient salts. The value of combining Nathansohn's and Brandt's views seems first to have been fully realized by Hentschel and Wattenberg in 1930 (61). The introduction of a reliable technique for determining phosphate in sea water by Atkins (3) provided an important impetus.

Taking all seas as a whole, it is now possible to state, approximately at least, that the replenishment of nutrients in the productive layers is normally the essential factor that determines the magnitude of the annual organic production. The replenishment may be due to regeneration *in situ* or to upwelling and turbulence whereby nutrient-rich subsurface water is carried into the photic zone.

Nutrient salts.—Ketchum, *et al.* (71) have recently reviewed the literature on the mineral nutrition of phytoplankton and the productivity in relation to nutrients. Little has been added to our knowledge during recent years. It is, however, becoming more and more evident that it is inadequate to compare "weeds" like *Chlorella* with the algae found ordinarily in oligotrophic habitats (102, 139, 141). Whereas in the laboratory it is possible to create nutrient-deficient cells (showing for example a rate of respiration nearly as high as light-saturated photosynthesis) in nature both grazing and sinking will normally prevent continuance of a nongrowing population. If the supply of nutrient salts is reduced in nature, the original population of algae will soon disappear, being replaced by a small population of algal species adapted to the scantier supply. The size of a given population seems to be maintained primarily by the balance between the zooplankton and the phytoplankton. This balance again matches the supply of nutrients (135).

Replenishment of nutrients in the open sea is provided principally by water circulation (61, 144) with nutrient-rich subsurface water being carried up into the photic layer. It is hardly possible at present to experimentally investigate regeneration of the nutrient salts in the open water. If sea water is enclosed in bottles, the growth of bacteria completely changes it (161).

In contrast to the open ocean the replenishment of the nutrient salts in shallow coastal areas seems to be primarily by regeneration at the site. This regeneration very likely does not take place mainly in the free water masses but in the top layer of the bottom sediments. The highest rate of production is found in areas without any pronounced stratification of the water masses (50, 98, 116, 129). The water masses of the photic zone must be in more or less continuous contact with the bottom sediment.

The rate of production in shallow water areas seems to always closely depend on temperature. In the tropics such localities thus are normally very productive (140). In temperate latitudes the curves showing annual variation of temperature and primary production generally are very similar (50, 129, 137). For a Danish coastal water it has been possible over a period of years to correlate the mean temperature during the summer with the rate of primary production during the same time (137). Most likely the microbiological processes taking part in the mineralization of the organic sediments are temperature dependent, thus indirectly giving rise to a higher primary production.

Light.—Light provides the energy necessary for the plankton algae to transform inorganic matter into organic matter but only the light absorbed by the pigments active in photosynthesis is used for this transformation.

Generally only a small part of the light absorbed by the sea is absorbed by these pigments, with the major portion being absorbed by the water itself or by inorganic or organic particles and by dissolved matter. In the ocean, far from the influence of the coast, the absorption by the water molecules is ordinarily the most important. In the deep parts of the oceans—the most unproductive areas—light absorption is practically identical with that theoretically to be expected in an "ocean" of absolutely pure water according to calculations made by Jerlov (cf. 140).

The rate of organic productivity per surface unit is, as predicted theoretically, inversely proportional to the depth of the photic layer. It is most obvious in the open ocean outside the influence of the coast (140) but it has been shown to be true even in freshwater lakes. Jerlov (66) presented a comprehensive survey of submarine light in the open ocean. The light dependency of photosynthesis in plankton algae will not be treated separately in the present review. A survey of the special problems concerning light and organic production in the sea has been reviewed by Steemann Nielsen (138).

Curves showing the rate of photosynthesis in natural populations of phytoplankton as a function of light intensity have been presented (38, 107, 126, 140, 146, 150). Steemann Nielsen and Hansen (142) have presented such curves covering habitats from the arctic, the temperate zone and the tropics, from the surface and from the lower part of the photic zone, from areas with vertically stabilized water masses and from areas lacking such a stabilization. In the temperate zone variations according to the seasons were also included. The rates of the enzymatic partial processes were shown to adjust themselves according to the light conditions ordinarily found at the habitat.

Talling (147) has presented a widely applicable expression for the total or integral photosynthesis of a natural population beneath a unit area of surface. The integral photosynthesis was shown to be related to the logarithm of the surface light intensity over a wide range of conditions. Depth profiles of primary production with a homogeneous vertical distribution of algae normally show a pronounced light inhibition near the surface during bright days in summer, as found by most workers. The layer showing the highest rate of production is ordinarily found at a depth where the light intensity is reduced by a factor of 2 to 3. The light inhibition near the surface is the main reason that variations in the daylight reaching the surface have only a relatively small influence on the total photosynthesis beneath a unit area of surface (6, 131, 140, 147). For the top layer of the photic zone during a bright day Rodhe (103) has shown that the sum of five short-time exposures is higher than the result from a bottle exposed the whole day. The influence of light inhibition is cumulative.

Doty & Oguri (36), and Shimada (115) have given evidence for a diurnal fluctuation in the rate of light-saturated photosynthesis in tropical waters. It coincides with a similar fluctuation in the concentration of chlorophyll *a*. The nature of these fluctuations is still unknown. As the minima

both in chlorophyll and rate of light-saturated photosynthesis are found near sunset, a similarity may be assumed between these fluctuations and light inhibition. In temperate water Yentsch & Ryther (159) have shown that similar but slight fluctuations may be found.

In a natural population of plankton algae a light utilization as high as that found in a terrestrial population of higher plants on eutrophic soil seems impossible. Because of the special inner structure (anatomy) and the placing of the leaves, conditions in communities of higher plants are created whereby the light is "diluted" before reaching the chloroplasts. This dilution facilitates a better utilization of the light. In a community of plankton algae no such dilution takes place. Therefore the maximum quantity of chlorophyll per surface unit in the photic layer of a community of plankton algae is only about one-fifth that in a productive community of higher terrestrial plants and the maximum yield is considerably less (134).

A plankton alga has to follow the water masses carrying it. Ordinarily, but by no means always, the photic zone in the open ocean has a greater vertical extension than the layer in which the water masses are more or less vertically mixed daily. However, because of convection due to the cooling of the surface water and because of storms, a well mixed layer of considerable thickness develops at higher latitudes during winter. Vertical stabilization of the water masses is necessary before production can start again in such areas. Braarud & Klem (16) were the first to call attention to the influence of such a situation on the production of phytoplankton. Several other workers have provided further evidence. Sverdrup (143) tackled the problem theoretically. A population of phytoplankton can increase only if photosynthesis exceeds respiration and the losses due to grazing and sinking (49). With certain assumptions and simplifications it was possible for Sverdrup to compute the critical depth during the spring at the weather ship "M" in the Norwegian Sea.

Temperature.—The inadequacy of laboratory experiments as a means to understanding the effect of the important factors for the growth of plants in the sea is especially obvious when we consider temperature.

Temperature does not influence the rate of photosynthesis at low light intensities. The overall reaction is here limited by the rate of the photochemical partial process which is independent of temperature. At high light intensities the overall reaction is limited by the rates of the enzymatic partial processes which are temperature dependent. If the temperature increases by 10°C., the rates increase by a factor of 2 to 3. Working with cultures of unicellular algae this has been shown to be correct for all of the species investigated, if the temperature interval is kept within the limits in which the species are able to grow (89). When proposing a mathematical equation describing organic production in the sea, Riley *et al.* (101) introduced a temperature coefficient. Dietrich & Kalle (32) even consider temperature to be a major direct factor for organic production in the sea. Recent investigations have shown, however, that the direct influence of temperature on organic production in the sea is fairly insignificant. The strict

dependence of the production rate in shallow water localities on temperature is an indirect consequence of the regeneration of the nutrient salts.

Steemann Nielsen & Hansen (142) have shown that the effect of low temperature at the habitat is counteracted in the algae by an increase in the concentration of the different enzymes active in photosynthesis. In nature, plankton algae seem to be able to adjust their enzymes over a wide range to the level most appropriate for prevailing ecological conditions. This concerns not only the enzymes active in photosynthesis but also those active in respiration. The rates of these two processes match each other.

Grazing, sinking, and external metabolites.—Cushing (30, 31) recently presented reviews on the effect of grazing in reducing primary production. It is thus unnecessary to present details here. The references (56, 101, 140) may just be mentioned as dealing with the problems of sinking and grazing. The influence of external metabolites has been reviewed by Lucas (82, 83).

Mathematical models of the causal relations in production have been presented by some investigators. The results have been most satisfactory where the worker restricted himself to problems of a limited extent, such as Fleming (40) and Steele (122).

Addenda.—It has been impossible for the author, who does not read Russian, to discuss all Russian articles. Fortunately (10, 39, 118, 157) present surveys in English or French. Besides articles discussed in the present review (8, 11, 117, 151, 155, 156, 158) may also be mentioned.

LITERATURE CITED

1. Angot, M., Doty, M. S., and Oguri, M., *Inst. Franc. d'Oceanie, Sect., Ocean. Rappt. Sci.*, **4,** 1 (1958)
2. Atkins, W. R. G., *J. Marine Biol. Assoc. United Kingdom,* **12,** 717 (1922)
3. Atkins, W. R. G., *J. Marine Biol. Assoc., United Kingdom,* **12,** 119 (1923)
4. Atkins, W. R. G., and Parke, M., *J. Marine Biol. Assoc. United Kingdom,* **29,** 609 (1951)
5. Atkins, W. R. G., and Jenkins, P. G., *Nature,* **177,** 1218 (1956)
6. Bauer, G. (*Inaugural-Dissertation,* München, Germany, 1957)
7. Berge, G., *Rappt. cons. explor. mer,* **144,** 85 (1958)
8. Bernard, F., *Ann. inst. Oceanog.,* **17,** 349 (1938)
9. Bernard, F., *Rappt. cons. explor. mer,* **144,** 103 (1958)
10. Bogorov, B. G., *Rappt. cons. explor. mer,* **144,** 117 (1958)
11. Bogorov, B. G., and Beklenishev, C. V., *Doklady Akad. Nauk. S.S.S.R.,* **104,** 141 (1955)
12. Bogoyavlenskii, A. N., *Trudy Inst. Okeanol. Akad. Nauk. S.S.S.R.,* **12,** 161 (1955)*
13. Braarud, T., *Hvalrådets Skrifter Norske Videnskaps-Akad. Oslo,* **10,** 1 (1935)
14. Braarud, T., *Hvalrådets Skrifter Norske Videnskaps-Akad. Oslo,* **28,** 1 (1945)
15. Braarud, T., *Rappt. cons. explor. mer,* **144,** 17 (1958)
16. Braarud, T., and Klem, A., *Hvalrådets Skrifter Norske Videnskaps-Akad. Oslo,* **1,** 1 (1931)

16a. Brandt, K., *Wiss. Meeresunters.,* **6,** 23 (1902)

17. Von Brand, T., *Biol. Bull.*, **69**, 221 (1935)
18. Brouardel, J., and Rinck, E., *C.R. Acad. Sci.*, **243**, 1797 (1956)
19. Brouardel, J., and Rinck, E., *J. conseil, Conseil permanent intern. exploration mer*, **24**, 10 (1958)
20. Buch, K., *Rappt. cons. explor. mer*, **132**, 36 (1952)
21. Buch, K., and Gripenberg, S., *HavsforsknInst. Skr., Helsingf.*, **118** (1938)
22. Cooper, L. H. N., *J. Marine Biol. Assoc. United Kingdom*, **18**, 729 (1932)
23. Cooper, L. H. N., *J. Marine Biol. Assoc. United Kingdom*, **18**, 677 (1933)
24. Cooper, L. H. N., *Rappt. cons. explor. mer*, **144**, 35 (1958)
25. Corlett, J., *Rappt. cons. explor. mer*, **144**, 76 (1958)
26. Currie, R. J., *Rappt. cons. explor. mer*, **144**, 96 (1958)
27. Cushing, D. H., *Ministry of Agric. Fisheries and Food Fisheries Inv. Ser. 2*, **8**(7), 1 (1955)
28. Cushing, D. H., *Rappt. cons. explor. mer*, **144**, 32 (1958)
29. Cushing, D. H., *Rappt. cons. explor. mer*, **144**, 73 (1958)
30. Cushing, D. H., *Rappt. cons. explor. mer*, **144**, 149 (1958)
31. Cushing, D. H., *J. conseil, Conseil permanent intern. exploration mer*, **24**, 455 (1959)
32. Dietrich, G., and Kalle K., *Allgemeine Meereskunde. Eine Einführung in die Ozeanographie* (Berlin-Nikolassee, 1957)
33. Digby, P. S. B., *J. Animal Ecol.*, **22**, 289 (1953)
34. Doty, M. S., Oguri, M., and Pyle, R., *Abstr. Proc. Hawaiian Acad. Sci.* 1955-1956, **20** (1956)
35. Doty, M. S., and Oguri, M., *J. conseil, Conseil permanent intern. exploration mer*, **22**, 33 (1956)
36. Doty, M. S., and Oguri, M., *Limnol. & Oceanog.*, **2**, 37 (1957)
37. Doty, M. S., and Oguri, M., *Rappt. cons. explor. mer*, **144**, 47 (1958)
38. Edmondson, W. T., *Ecology*, **37**, 161 (1957)
39. Fedosov, M. V., *Rappt. cons. explor. mer*, **144**, 61 (1958)
40. Fleming, R. H., *J. conseil, Conseil permanent intern. exploration mer*, **14**, 210 (1939)
41. Fogg, G. E., *Rappt. cons. explor. mer*, **144**, 56 (1958)
42. Gaarder, T., and Gran, H. H., *Rappt. cons. explor. mer*, **42**, 3 (1927)
43. Gessner, F., *Arch. Hydrobiol.*, **40**, 687 (1944)
44. Gillbricht, M., *Kieler, Meeresf.*, **8**, 173 (1952)
45. Goldberg, E. D., Baker, M., and Fox, D. L., *J. Marine Research*, **11**, 194 (1952)
46. Graham, H. W., *J. Marine Research*, **5**, 153 (1943)
47. Gran, H. H., *Rappt. cons. explor. mer*, **56**, 1 (1929)
48. Gran, H. H., *Avhandl. Norsk. Videnskaps-Akad. Oslo, I, Mat. Naturv. Kl.*, **5**, 1 (1930)
49. Gran, H. H., and Braarud, T., *J. Biol. Board of Can.*, **1**, 279 (1935)
50. Grøntved, J., and Steemann Nielson, E., *Medd. Komm. Danm. Fisk. Havund. Plankton*, **5**, 1 (1957)
51. Guillard, R. R. L., Doty, M. S., and Oguri, M., *Abstr. Proc. Hawaiian Acad. Sci.*, 1955–56, **10** (1956)
52. Halldal, P., *Hvalrådets Skrifter Norske Videnskaps-Akad. Oslo*, **38**, 1 (1953)
53. Hanaoka, T., *Bull. Naikai Reg. Fish. Res. Lab.*, **8**, 1 (1955)
54. Hanaoka, T., *Rappt. cons. explor. mer*, **144**, 28 (1958)
55. Hart, T. J., *Discovery Repts.*, **21**, 261 (1942)
56. Harvey, H. W., *J. Marine Biol. Assoc. United Kingdom*, **29**, 97 (1950)

57. Harvey, H. W., Cooper, L. H. N., Lebour, M. V., and Russel, F. S., *J. Marine Biol. Assoc. United Kingdom*, **20**, 407 (1935)
58. Hensen, V., *Ergeb. Plankton-Exped. Humboldt-Stiftung*, **5**, 1 (1911)
59. Hentschel, E., *Wiss. Ergeb. Deutsch. Atlan. Exped. Forsch. Vermessungssch. "Meteor" 1925–1927*, **10**, 1 (1932)
60. Hentschel, E., *Wiss. Ergeb. Deutsch. Atlan. Exped. Forsch. Vermessungssch. "Meteor" 1933–1936*, **11**, 1 (1933-36)
61. Hentschel, E., and Wattenberg, H., *Ann. Hydrol. Mar. Meteor*, **58**, 273 (1930)
62. Holmes, R. W., *Rappt. cons. explor. mer*, **144**, 109 (1958)
63. Holmes, R. W., Schaefer, M. B., and Shimada, B. M., *Bull. Inter-Am. Trop. Tuna Comm.*, **2** (4), 129 (1957)
64. Ichimura, S., and Saijo, Y., *Botan. Mag. (Tokyo)*, **72**, 193 (1959)
65. Jenkins, P. G., *Deep-Sea Research*, (Suppl.), **3**, 58, (1955)
66. Jerlov, N. G., *Rept. Swed. Deep-Sea Exped.*, **3**, 1 (1951)
67. Jerlov, N. G., *Rept. Swed. Deep-Sea Exped.*, **3**, 71 (1953)
68. Jitts, H. R., *C.S.I.R.O., Research Rev., Rep. Div. Fish. & Oceanog.*, **8**, 1 (1957)
69. Jitts, H. R., and Rotschi, H., *Radioisotopes in Scientific Research*, **4**, 607; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
70. Kalle, K., *Deut. Hydrograph. Z.*, **1**, 2 (1948)
71. Ketchum, B. H., Ryther, J. H., Yentsch, C. S., and Corwin, N., *Rappt. cons. explor. mer*, **144**, 132 (1958)
72. King, J. E., Austin, T. S., and Doty, M. S., *U.S. Fish & Wildlife Serv. Spec. Sci. Rept. Fisheries Ser.*, **201**, 155 (1957)
73. Kreps, E., and Verjbinskaya, N., *J. conseil, Conseil permanent intern. exploration mer.*, **5**, 329 (1930)
74. Kreps, E., and Verjbinskaya, N., *J. conseil. Conseil permanent intern. exploration mer*, **7**, 25 (1932)
75. Krey, J., *J. conseil, Conseil permanent intern. exploration mer.*, **14**, 201 (1939)
76. Krey, J., *Kieler Meeresforsch.*, **7**, 58 (1950)
77. Krey, J., *Kieler Meeresforsch.*, **8**, 16 (1951)
78. Krey, J., *Kieler Meeresforsch.*, **12**, 46 (1956)
79. Krey, J., *Rappt. cons. explor. mer*, **144**, 20 (1958)
80. Lohmann, H., *Komm. z. Wiss. Unters. Deutsch. Meere in Kiel Biol. Anst. Helgoland, Wiss. Meeresunters. N.F.Abt.Kiel*, **10**, 131 (1908)
81. Lohmann, H., *Arch. Biontol. Berl.*, **4**, 1 (1920)
82. Lucas, C. E., *Biol. Revs. Cambridge Phil. Soc.*, **22**, 270 (1947)
83. Lucas, C. E., *Rappt. cons. explor. mer*, **144**, 155 (1958)
84. Marshall, N., *J. Marine Research*, **15**, 14 (1956)
85. Marshall, P. T., *J. conseil, Conseil permanent intern. exploration mer*, **23**, 173 (1958)
86. Miyake, Y., *Radioisotopes in Scientific Research*, **4**, 651; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
87. Miyake, Y., Sugiura, Y., and Saruhashi, K., *Papers Meteorol. and Geophys. (Tokyo)*, **5**, 89 (1954)
88. Nathansohn, A., *Intern. Rev. ges. Hydrobiol. und Hydrogr.*, **1**, 37 (1908)
89. Rabinowitch, E., *Photosynthesis and Related Processes*, **I–II** (Interscience Publ., New York, 1945–56)
90. Redfield, A., *Papers Phys. Oceanog. Meteorol., Mass. Inst. Technol., and Woods Hole Oceanog. Inst.*, **9 (2)**, 1942

91. Richards, F. A., and Thompson, Th., *J. Marine Research,* **11,** 156 (1952)
92. Riley, G. A., *J. Marine Research,* **1,** 335 (1938)
93. Riley, G. A., *J. Marine Research,* **2,** 145 (1939)
94. Riley, G. A., *Bull. Bingham Oceanog. Coll.,* **7** (3), 1 (1941)
95. Riley, G. A., *Bull. Bingham Oceanog. Coll.,* **7** (4), 1 (1941)
96. Riley, G. A., *J. conseil, Conseil permanent intern. exploration mer,* **19,** 85 (1953)
97. Riley, G. A., *Deep-Sea Research,* (Suppl.), **3,** 224 (1955)
98. Riley, G. A., *Bull. Bingham Oceanog. Coll.,* **15,** 324 (1956)
99. Riley, G. A., *Limnol. & Oceanog.,* **2,** 252 (1957)
100. Riley, G. A., and Gorgy, S., *J. Marine Research,* **7,** 100 (1948)
101. Riley, G. A., Stommel, H., and Bumpus, D. F., *Bull. Bingham Oceanog. Coll.,* **12** (3), 1 (1949)
102. Rodhe, W., *Symbolae Botan. Upsalienses,* **10** (1), 1 (1948)
103. Rodhe, W., *Rappt. cons. explor. mer,* **144,** 122 (1958)
104. Rodhe, W., Vollenweider, R. A., and Nauwerck, A., in *Perspectives in Marine Biology* (Scripps Inst. of Oceanog., La Jolla, Calif., 299 pp., 1958)
105. Ryther, J. H., *Deep-Sea Research,* **2,** 134 (1954)
106. Ryther, J. H., *Limnol & Oceanog.,* **1,** 72 (1956)
107. Ryther, J. H., *Limnol. & Oceanog.,* **1,** 61 (1956)
108. Ryther, J. H., and Vaccaro, R. F., *J. conseil, Conseil permanent intern. exploration mer,* **20,** 25 (1954)
109. Ryther, J. H., and Yentsch, C. S., *Limnol & Oceanog.,* **2,** 281 (1957)
110. Ryther, J. H., and Yentsch, C. S., *Limnol & Oceanog.,* **3,** 327 (1958)
111. Schmidt-Ries, H., *Arch. Hydrobiol.,* **29,** 553 (1936)
112. Schroeder, H., *Naturwissenschaften,* **7,** 8 (1919)
113. Seiwell, H. R., *J. conseil, Conseil permanent intern. exploration mer,* **10,** 20 (1935)
114. Sesták, Z., *Preslia,* **30,** 138 (1958)
115. Shimada, B. M., *Limnol. & Oceanog.,* **3,** 336 (1958)
116. Smayda, T. J., *Limnol. & Oceanog.,* **2,** 342 (1957)
117. Smetanin, D. A., *Trudy Inst. Okeanol. Akad. Nauk. S.S.S.R.,* **17,** 192 (1956)*
118. Sorokin, J. I., *Radioisotopes in Scientific Research,* **4,** 633; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
119. Sorokin, J. I., and Kozlianinov, M. V., *Doklady Akad. Nauk S.S.S.R.,* **116,** 863 (1957)
120. Sorokin, J. I., and Coblentz-Mishke, O. Y., *Doklady Akad. Nauk. S.S.S.R.,* **122,** 1018 (1958)
121. Steele, J. H., *J. Marine Biol. Assoc. United Kingdom,* **36,** 233 (1957)
122. Steele, J. H., *Rappt. cons. explor. mer,* **144,** 79 (1958)
123. Steemann Nielsen, E., *Medd. Komm. Danm. Fisk. Havund., Plankton,* **2** (4), 1 (1932)
124. Steemann Nielsen, E., *J. conseil, Conseil permanent intern. exploration mer,* **8,** 201 (1933)
125. Steemann, Nielsen, E., *Medd. Komm. Danm. Fisk. Havund., Plankton,* **3** (1), 1 (1935)
126. Steemann Nielsen, E., *Medd. Komm. Danm. Fisk. Havund., Plankton,* **2** (4), 1 (1937)
127. Steemann Nielsen, E., *J. conseil, Conseil permanent intern. exploration mer,* **13,** 197 (1938)
128. Steemann Nielsen, E., *Medd. Komm. Danm. Fisk. Havund., Plankton,* **3** (4), 1 (1940)

129. Steemann Nielsen, E., *Medd. Komm. Danm. Fisk. Havund., Plankton*, **5** (4), 1 (1951)
130. Steemann Nielsen, E., *J. conseil, Conseil permanent intern. exploration mer*, **18**, 117 (1952)
131. Steemann Nielsen, E., *J. conseil, Conseil permanent intern. exploration mer*, **19**, 309 (1954)
132. Steemann Nielsen, E., *Physiol. Plantarum*, **8**, 945 (1955)
133. Steemann Nielsen, E., *Deep-Sea Research* (Suppl.), **3**, 281 (1955)
134. Steemann Nielsen, E., *Physiol. Plantarum*, **10**, 1009 (1957)
135. Steemann Nielsen, E., *J. conseil, Conseil permanent intern. exploration mer*, **23**, 178 (1958)
136. Steemann Nielsen, E., *Rappt. cons. explor. mer*, **144**, 38 (1958)
137. Steemann Nielsen, E., *Rappt. cons. explor. mer*, **144**, 92 (1958)
138. Steemann Nielsen, E., *Rappt. cons. explor. mer*, **144**, 141 (1958)
139. Steemann Nielsen, E., and Al Kholy, A. A., *Physiol. Plantarum*, **9**, 144 (1956)
140. Steemann Nielsen, E., and Aabye Jensen, E., *Galathea-Repts., Sci. Research of the Danish Deep-Sea Expedition Around the World, 1950–52*, **1**, 49 (1957)
141. Steemann Nielsen, E., and Hansen, V. K., *Deep-Sea Research*, **5**, 222 (1959)
142. Steemann, Nielsen, E., and Hansen, V. K., *Physiol. Plantarum*, **12**, 353 (1959)
143. Sverdrup, H. U., *J. conseil, Conseil permanent intern. exploration mer*, **18**, 287 (1953)
144. Sverdrup, H. U., *J. Marine Research*, **14**, 287 (1955)
145. Tamiya, H., *Ann. Rev. Plant. Physiol.*, **8**, 309 (1957)
146. Talling, J. F., *New Phytologist*, **56**, 1 (1957)
147. Talling, J. F., *New Phytologist*, **56**, 133 (1957)
148. Utermöhl, H., *Verhandl Int. Ver. Limnol.*, **5** (1931)
149. Vaccaro, R. F., and Ryther, J. H., *J. conseil, Conseil permanent intern exploration mer*, **20**, 18 (1954)
150. Verduin, J., *Ecology*, **37**, 40 (1957)
151. Vodjanitskii, V. A., *Conf. Fish. & Fish. Managem. Akad. Nauk. S.S.S.R. Ichth. Kom. Trudy Sov.*, **1**, 487 (1953)
152. Waldichuk, M., *J. Fisheries Research Board Can.*, **13**, 7 (1956)
153. Wattenberg, H., *Wiss. Ergeb. Deutsch, Atlantic. Exped. Forsch. Verm. Sch. "Meteor" 1925–1929*, **9**, 133 (1957)
154. Wimpenny, R. S., *Rappt. cons. explor. mer*, **144**, 70 (1958)
155. Winberg, G. G., *Priroda*, **5**, 92 (1954)
156. Winberg, G. G., *Zhur. Obscheĭ Biol.*, **17**, 364 (1956)
157. Winberg, G. G., *Rappt. cons. explor. mer*, **144**, 65 (1958)
158. Winberg, G. G., and Sivko, T. N., *Izvest. Akad. Nauk. Belorus, S.S.S.R.*, **3**, 61 (1953)
159. Yentsch, C. S., and Ryther, J H., *Limnol. & Oceanog.*, **2**, 140 (1957)
160. Yentsch, C. S., and Ryther, J. H., *J. conseil, Conseil permanent intern. exploration mer*, **24**, 231 (1959)
161. ZoBell, C. E., and Andersson, D. Q., *Biol. Bull.*, **71**, 324 (1936)

* English translation will be announced in Technical Translations, issued by the Office of Technical Services, U.S. Dept. of Commerce, and will be made available by the Photoduplication Service, Library of Congress and by the SLA Translation Center at the John Crear Library, Chicago, Illinois.

THE PRIMARY PROCESSES OF PHOTOSYNTHESIS IN PLANTS[1,2,3]

By A. A. Krasnovsky

A. N. Bakh Institute of Biochemistry, U.S.S.R. Academy of Sciences, Moscow, U.S.S.R.

In this review an attempt is made to consider the main lines of investigation in the field of the mechanism of photosynthesis.

The sequence of the presentation corresponds approximately to that of elementary processes in photosynthesis. The state of pigments in the organisms and photophysical convergences of the energy of a light quantum absorbed by the pigment system are considered first, followed by a study of the nature of the primary photochemical act. This is closely connected with the study of reversible chlorophyll transformations in solutions and in living photosynthetic organisms.

Subsequent reactions of primary photoproducts lead to the formation of more stable compounds that store the energy of light quanta; these reactions are discussed in the sections "Photosensitizing action of chlorophyll in hydrogen transfer" and "Involvement of photoproducts in the system of biochemical processes." Active compounds thus formed become involved in the system of dark enzymic reactions leading to carbon dioxide assimilation; a very brief presentation of the results of the study of intermediates by means of tracers closes the paper. Most attention in the review is given to the state and photochemical transformations of the pigments.

Different Forms of Pigments in Organisms

A study of the state of pigments in photosynthetic organisms is necessary in order to understand the nature of their participation in the reactions of photosynthesis.

The investigation of chloroplasts, grana, and chromatophores in bacteria by means of electron microscopy leads to a conclusion regarding their lamellar structure. These experiments, however, have not yet provided definite information on the arrangement and packing of pigment molecules.

[1] This review is based mainly on the papers that were available to the reviewer up to July 1, 1959, and had been issued between 1955 and 1959; therefore, the literature cited is not exhaustive.

[2] The following abbreviations will be used: DNP, dinitrophenol; DPN, diphosphopyridine nucleotide; DPNH, diphosphopyridine nucleotide (reduced form); ESR, electron spin resonance; FMN, flavin mononucleotide; TPNH, triphosphopyridine nucleotide (reduced form).

[3] In many cases, referring to the works of the laboratory to which this reviewer belongs, the expressions "in our laboratory" and "in our group" will be used to avoid repeating the same names.

This area of investigation is covered in Wolken's review (1). Use of spectral technique enabled us to draw the conclusion that chlorophyll in plants can be present in various forms of aggregation and linkage with proteins and lipids. The present discussion will be directed to comparing spectral properties of pigments in the organisms with those of individual pigments in different known states.

Bacteriochlorophyll.—Wassink, Katz & Dorrenstein (2) and French (3) have found, in various strains of purple bacteria, three absorption maxima of bacteriochlorophyll in the near infrared at 800, 850, and 890mμ, but the pigment extracted from the cell with organic solvents has only one maximum at 770 to 780 mμ. The fluorescence maximum of purple bacteria lies between 910 to 920 mμ (4, 5), while that of bacteriochlorophyll in a solution lies at about 780 to 790 mμ. Exposure to 60°C. (which leads to protein denaturation) and the action of proteolytic enzymes do not markedly affect the shape of the absorption spectrum. Heating up to 80 to 90°C. results in the decrease of the maximum at 890 mμ, the peak representing the least stable form. Prolonged exposure to 100°C. leads to a gradual shift of the maximum to 760 to 770 mμ (6). When pyridine is gradually added to a water suspension of bacteria, the maximum at 890 mμ is the first to disappear, followed by that at 850 mμ (7). Centrifugation of a bacterial homogenate at 150,000 g after treatment with a 0.5 per cent detergent solution brings about a prevailing sedimentation of the form with the maximum at 890 mμ (8).

To judge the properties of different forms, we studied the kinetics of their irreversible (destructive) bleaching. (A discussion of the reversible bleaching is presented below.)

Upon intensive illumination in the air, the form of bacteriochlorophyll with the maximum at 890 mμ is the first to bleach (7). This is in accordance with the observations of Duysens (9, 9a) and Goedheer (10).

Absorption spectra of bacteriochlorophyll isolated in pure state from cultures of photosynthetic bacteria by means of sugar chromatography were studied in our laboratory (11, 11a), as well as by Weigl (12), and by Holt & Jacobs (13). Bacteriochlorophyll solutions (extracts) have the absorption maximum in the region of 770 to 780 mμ. Thus, the interaction with polar molecules of the solvent does not affect the spectral shifts found in living cells. Our group (6) pointed out that aggregated forms of bacteriochlorophyll in solid films (obtained by evaporating pure bacteriochlorophyll solutions in vacuum) and in aqueous colloidal solutions show a far shift to the infrared (with absorption maxima at 790, 800, and 850 to 880 mμ) as found in living bacteria (6). Similar results were obtained in the experiments of Jacobs, Vatter & Holt (14) which showed crystalline bacteriochlorophyll to have its absorption maximum at 865 mμ. The experiments of Komen (15) reproduced our data on the absorption spectra of bacteriochlorophyll colloidal solutions. Bacteriopheophytin, unlike bacteriochlorophyll, gives one type of packing with the maximum at 850 mμ (6).

Effective interaction between π-electron systems of closely arranged pigment molecules results in a considerable distortion of the absorption spectrum. Different types of packing, with different absorption maxima, seem to be associated with a different orientation of pigment molecules and with the formation of solvent molecule "bridges" probably linking pigment magnesium atoms in aggregated structures.

A weak fluorescence of bacteriochlorophyll solid films at minus 150°C. was noticed in our experiments in the region of 910 to 920 mμ (16). It is the form "890" which seems to fluoresce.

Bacterioviridin (chlorobium-chlorophyll).—Katz & Wassink (17) and Larsen (18) noticed that green sulfur bacteria have their absorption maximum at 740 mμ while the pigment extracted in a solution has one at 660 to 670 mμ.

In water-glycerine mixtures, green bacteria show also a weak maximum at 670 mμ which probably corresponds to the "monomeric" form of the pigment (19, 19a). A weak maximum at 680 mμ and an intensive one at 760 to 780 mμ are to be found in the fluorescence spectrum of a green bacteria suspension. The "monomeric" maximum at 680 mμ increases in the presence of glycerine.

The absorption of bacterioviridin, both in solutions extracted from bacteria and in a purified state, is similar to that of chlorophyll-*a*. Our experiments (19) showed that bacterioviridin solid films, obtained by the evaporation of ether solutions in vacuum, have their maximum at 730 to 740 mμ, which corresponds to pigment absorption in living bacteria. The pigment seems to be easily crystallized. Aqueous colloidal solutions of bacterioviridin give two types of packing with the maxima at 710 to 720 mμ and 670 to 680 mμ, respectively. Our experiments revealed fluorescence of solid films of pure bacterioviridin with the maximum at 760 to 780 mμ, i.e., in the same region as that of living bacteria (19). All the data obtained show most of the pigment in green sulfur bacteria to be present in regularly arranged aggregated forms, while a small part occurs in "monomeric" forms.

Protochlorophyll.—It is necessary to study the state of the chlorophyll precursor in order to understand the state of chlorophyll-*a* in plants. Monteverde and Lyubimenko, at the start of this century, made the observation that protochlorophyll in the inner coats of Cucurbitaceae seeds could not be transformed to chlorophyll upon illumination and thus differed from protochlorophyll of etiolated leaves.

Smith, *et al.* (20) and Shibata (21), by measuring absorption spectra of etiolated leaves, found two protochlorophyll forms with the maxima at 635 and 648 mμ, respectively. The latter active form rapidly undergoes transformation to chlorophyll when illuminated. Our group (22), by measuring fluorescence spectra of etiolated leaves frozen to minus 150°C., revealed two forms of protochlorophyll with fluorescence maxima at 635 and 655 mμ. Upon illumination, the 655 mμ form was rapidly transformed to the

chlorophyll precursor. If the form with maximum absorption at 648 mμ corresponds to that fluorescing at 655 mμ, the fluorescence at 635 mμ may correspond to another fluorescent form of protochlorophyll with the absorption at 625 to 630 mμ, which peak would seem to be concealed by the general shape of the maximum observed at 635 mμ. Thus, the results of these independent experiments of both laboratories show the existence of pigment forms that differ in their photobiological activity.

When grinding etiolated bean leaves in a phosphate buffer at a pH of 8.5, protochlorophyll that is bound with proteins preserves its ability to be transformed to chlorophyll upon illumination (23, 24). While measuring the fluorescence spectra of homogenates of bean leaves grown in the dark, our group found both an active and an inactive form of protochlorophyll (25). Upon illumination of homogenates of etiolated leaves, a form of chlorophyll with a fluorescence maximum at 690 mμ and then at 680 mμ is formed. This form does not, however, transit to the maximum of 686 mμ (characteristic of green leaves), as no chlorophyll accumulation occurs under these conditions. Protochlorophyll formation was observed by our group (26) not only in etiolated but in green leaves as well, by means of measuring fluorescence spectra at low temperatures. Having kept green sugar beet leaves in the dark, we succeeded in observing the appearance of fluorescence spectra of protochlorophyll that is transformed to chlorophyll upon illumination of green leaves (26). Smith *et al.* obtained active preparations extracting "protochlorophyll-holochrome" with glycerine (27) and made attempts to isolate it by precipitation with ammonium sulfate and centrifugation. Purified preparations with their absorption maxima at 636 mμ seemed to be a mixture of active and inactive forms (28). Their molecular weight was within the range of 1.2×10^6 to 2.1×10^6.

Measuring the quantum yield of chlorophyll formation from protochlorophyll gave the value of 0.6 (29). Measuring fluorescence polarization in protochlorophyll-holochrome led to the conclusion that polarization in bound protochlorophyll is equal to that of protochlorophyll dissolved in castor oil (30); thus "holochrome" is the "monomeric" form.

Godnev & Shlyck investigated the nature of the primary products of the photochemical reaction occurring upon illumination of etiolated leaves (31). Participation of enzymic processes was eliminated by means of preliminary cooling. Besides the pigment resembling chlorophyllid by its spectrum and solubility in alkali previously found by Wolf & Price (32), and Löeffler (33), chlorophyll-*a* was observed to appear at the very beginning of the process. Godnev and Shlyck reached the conclusion that there are pigments both with and without phytol in the protochlorophyll of etiolated leaves.

Chlorophyll: changes of state in the course of its formation upon greening of etiolated leaves.—Chlorophyll freshly formed from protochlorophyll was found by our group to have its absorption maximum at 670 mμ in leaf homogenates, while this maximum gradually shifted up to 678 mμ during

greening (23, 24). Bleaching of chlorophyll in homogenates of both greening and green leaves decreases with its accumulation; primarily formed chlorophyll bleaches rapidly in the presence of air. With chlorophyll accumulation, the absolute amount of the bleaching part slowly increases, being close to the initial value (34).

Further study of intermediate stages of chlorophyll formation from protochlorophyll was carried out by Shibata (21). He observed, in greening etiolated leaves, intermediate chlorophyll with the maximum at 684 mμ formed from protochlorophyll, which rapidly underwent conversion to chlorophyll "670," and then to chlorophyll "677."

Fluorescence spectra measurements at low temperature, carried out in our laboratory (22), revealed the formation of an intermediate chlorophyll form with a fluorescence maximum at 687 to 690 mμ that rapidly undergoes conversion to a chlorophyll with a fluorescence maximum at 680 mμ. Then, with chlorophyll accumulation, the maximum shifts to 686 mμ, the shift being, presumably, determined by the reabsorption of fluorescence.

Thus, forms of the pigment with absorption maxima at 670, 684, and 677 mμ, and with corresponding fluorescence maxima at 680, 690, and 686 mμ were revealed in the course of chlorophyll formation in greening etiolated leaves. Upon extraction with organic solvents, only one form, chlorophyll-*a*, was revealed at all stages. Thus these forms seem to differ in the type of linkage with proteins and lipids, though their exact chemical differences are in need of elucidation.

Chlorophyll states in photosynthetic organisms: absorption spectra.—Long ago in leaves and chloroplasts absorption bands were observed that corresponded to chlorophyll-*a* (at 675 to 680 mμ) and chlorophyll-*b* (about 650 mμ). In organisms containing only chlorophyll-*a* the maximum was found within the range of 675 to 680 mμ; only in the deep-water red alga *Phyllophora,* were maxima found at 690, 675, and 670 mμ (35). Under peculiar conditions French observed absorption in *Euglena* at 690 to 695 mμ (36, 36a).

The position of the chlorophyll absorption maximum in homogenates of sugar beet leaves undergoes alteration in relation to pH of the buffer solution (37, 38). In more alkaline media, the absorption maximum shifts to the shorter region corresponding to the greatest disaggregation (672 mμ at pH of 10). In more acid media it shifts to the long-wave side (678 mμ at pH of 4). In certain conditions of homogenate preparation (pH of 8.6) a part of the chlorophyll lipoprotein seems to disaggregate.

Under the usual spectrophotometric procedure there is expressed the relation of optical density to the wave length [$D = f(\lambda)$]. French & Church (39) made an apparatus of a new type for "derivative spectrophotometry" in which $dD/d\lambda = f(\lambda)$ is recorded. This mode of measurement reveals those maxima that under the usual mode of measurement are concealed by the asymmetrical contour of the main absorption band. Application of this technique enabled French & Huang (40) to observe in

absorption spectra of *Chlorella* and other organisms 2 to 3 maxima in the region of 670 to 690 mμ. These seem to correspond to different forms of chlorophyll-*a*.

State of chlorophyll: fluorescence spectra.—When measuring fluorescence spectra of green organs of high chlorophyll concentration, the position of the true main "red" fluorescence maximum of chlorophyll is distorted as a result of reabsorption (41); the second and third fluorescence maxima (about 730 mμ and 815 mμ) do not undergo this distortion. These errors diminish with decrease of the optical path of fluorescence light from the interior of the leaf. Therefore, reabsorption is less in the case of leaves infiltrated by water and also with fluorescence excitation by more strongly absorbed light which penetrates less into the tissue. With the use of tissues containing little chlorophyll (mutants, *aurea* forms) there always exists the possibility that the state of this pigment differs from its state in the dark green tissue.

In fluorescence spectra of leaves and algae one usually finds maxima at 686, 730 to 735, and 812 to 815 mμ. In a number of cases, along with these common bands, our group could observe in green leaves a maximum at 690 mμ, which probably corresponds to an intermediate form of chlorophyll (22). In leaves frozen to liquid nitrogen temperature there are clearly seen maxima at 686 and 690 mμ which have a fine structure (26). It is likely that the ratio of the forms of chlorophyll depends on a number of physiological conditions, as well as on temperature. Perhaps, this may account for the Emerson effect (42, 42a) on the shift of the long wavelength limit of photosynthesis. Illumination of organisms with red or blue light is also likely to bring about the appearance of newly formed chlorophyll participating in photosynthesis. This fact has to be taken into consideration when interpreting the results of quantum yield measurements of photosynthesis in red algae as in the experiments of Yocum & Blinks (43), and Brody (44).

Action of heat and solvents.—Heating usually leads to an absorption shift to the short region of the spectrum and to a corresponding shift of the fluorescence. Addition of organic solvents (alcohol, acetone, pyridine) to aqueous homogenates of chloroplasts or grana in amounts exceeding 50 per cent, usually results in the extraction of pigments into the solution. Small amounts of solvents (from 1 to 10 per cent), without extracting pigments, bring about a disturbance of the natural state of pigments that is manifested in the shift of absorption spectrum maximum from 678 mμ to 670 to 672 mμ and in a corresponding change of fluorescence. The action of pyridine is the most efficient (45). The works of Godnev and collaborators showed that a part of the chlorophyll can be extracted from leaves and isolated chloroplasts with petroleum ether (46, 47). When gradually extracting chlorophyll from the preparations of dried chloroplasts with the use of polar and nonpolar solvents, Ossipova found that 15 to 20 per cent of the pigments are extracted with petroleum ether (48). The author suggests that the extractable part of chlorophyll is unbound to protein, being dissolved

in lipids. The amount of the form of chlorophyll extracted with nonpolar solvents depended on the plant species (46, 47), increasing both with the age of the plant and with tripsin digesting the protein stroma of chloroplasts (48).

As mentioned above, different forms of pigments possess different resistances to destructive photochemical oxidation. The kinetics of chlorophyll bleaching in tissue homogenates of various plants were taken under study by our group (37, 38, 49).

A small part of chlorophyll bleaches particularly rapidly; the size of the rapidly bleaching fraction depends on the plant species, its physiological condition, and age; the largest amount of photochemically active (rapidly bleaching) form was found in sugar beet, *Chenopodium,* etc., while a very small amount was found in cereals (49). It is likely that this effect characterizes the disaggregation of chlorophyll-lipoprotein compounds in a buffer solution. Brown & French (50) studied bleaching of *Chlorella* homogenates by means of derivative spectrophotometry (50). Sapozhnikov & Maslova (51) showed that the thermolability of the chlorophyll-protein complex differed in different plants and underwent changes during the process of vegetation. Godnev & Akulovich (52), studying spectral changes upon exposing leaves to heat, found that these changes arise from protein denaturation and differ in plants of different types and ages.

Our group made a suggestion that different forms of pigments might be associated with different cell structures and tried to separate them by means of centrifugation at 25,000 g after cooling. It turned out that in leaf homogenates containing a considerable amount of the rapidly bleaching form, the absorption maximum shifted after centrifugation (in supernatant) from 677 mμ to 672 mμ, towards more disaggregated forms.

Different forms of pure chlorophyll.—It is known that no shift of the absorption maximum observed in plants can be found in chlorophyll solutions in organic solvents, or under the action of mild acids and bases. In strong bases however, the cyclopentanone ring becomes disrupted leading to an absorption shift to the short wavelength region; in strong acid media magnesium is replaced by hydrogen.

The study of chlorophyll monolayers on water, undertaken by Rabinowitch's group (53), showed the position of the absorption maximum to depend on the monolayer density, the degree of "bidimensional aggregation." The absorption maximum of "liquid," irregular chlorophyll monolayers was found at 675 mμ, while those of dense crystalline ones occurred at 730 mμ. Trurnit & Colmano (54) showed that at the water-oil boundary, the chlorophyll absorption maximum shifted from 672 to 680 mμ, depending on the density of monolayer packing from 8.10^3 to 16.10^3 mol./cm.^{2}. In dense layers the ratio of optical density in blue to that in red absorption maxima corresponds to the ratio observed in aqueous colloidal solutions of chlorophyll.

It was known long ago that colloids were formed upon adding chlorophyll solutions in organic solvents (which are miscible with water) to an excess of water. Freshly formed colloids have an absorption maximum at 670 mμ, which

shifts ever farther to infrared with ageing and further aggregation. When adding acetone solutions of chlorophyll to alkaline phosphate buffer solutions, disaggregated forms with a maximum at 670 mμ are formed, and in more acid media larger particles (maximum about 690 mμ at pH of 4.3) are formed that seem to possess crystalline structures (38, 55). Rabinowitch *et al.* (56) showed with ethylchlorophyllid-*a* that, with increase of particle size, the maximum reaches up to 740 mμ, corresponding to the crystalline form of the pigment (14, 56, 57). Crystalline chlorophyll-*a* with an absorption maximum at 730 mμ was obtained under peculiar conditions, in the presence of water, and was studied by means of x-ray diffraction (57 to 59). Under similar conditions, crystalline chlorophyll with the absorption maximum at 740 mμ was obtained by Zill, Colmano & Trurnit (60).

Our group obtained one more form of crystalline chlorophyll-*a* and -*b* with absorption maxima at 675 and 690 mμ respectively (55). Electron-diffraction study showed crystalline structure of aggregated forms of chlorophyll with the absorption maxima at 690 and 740 mμ. [The crystals have acicular form under the electron microscope (55).] The forms with the maximum at 670 to 675 mμ showed a weak regularity. It should be noted that crystallization of chlorophyll from the mixture of pyridine and dioxane [under the conditions suggested by Takashima (61) for the isolation of chlorophyll-lipoprotein] leads to the formation of crystals with "dioxane" packing and with the absorption maximum at 690 mμ. Brody (61a) observed that some types of aggregated forms of chlorophyll could possess fluorescence in the region of 715 to 720 mμ at liquid nitrogen temperature; perhaps this phenomenon is partly due to red fluorescence maximum reabsorption in frozen media having high scattering.

Chlorophyll-protein compounds.—Thus far the attempts to isolate a chlorophyll-protein compound of a definite composition have been unsuccessful. Only a paper of Takashima (61) described the isolation of crystals of "chlorophyll-lipoprotein" from a solvent mixture of α-picoline and dioxane. In view of its importance, this work attracted attention and was reproduced. Our group concluded that Takashima's crystals were chlorophyll crystals with "dioxane" packing which precipitated, together with proteins and lipids, under the conditions of the experiments (62). This is proved by the following observations: (*a*) under described conditions of crystallization, the "pure" chlorophyll gives the same crystals with the same absorption maximum as the "chlorophyll-lipoprotein"; (*b*) upon subsequent repeated crystallization the crystals are gradually depleted in protein without losing their form; (*c*) upon ultracentrifugation of chloroplast substance in 50 per cent pyridine, most of the chlorophyll remains in the low molecular weight fraction.

Formation and destruction of chlorophyll.—In this review we can not cover the extensive material devoted to chlorophyll biosynthesis, though the question of the rate of its formation and destruction in the organism is

closely related to the problem of the state of the pigment and its participation in photosynthesis.

Godnev & Shlyck observed a chlorophyll radioactivity decrease in plants previously given $C^{14}O_2$ and then carried to a label-free medium. These experiments proved the renovation of chlorophyll and, in particular, the destruction of some of its molecules even during the pigment accumulation in growing plants (63). Specific activity of chlorophyll-*a* during the renovation process is considerably higher than that of chlorophyll-*b*. This is true for both the phorbinic and phytolic parts of the molecule. Having studied the character of the process in time the above authors think it most likely that during biosynthesis in green leaves, chlorophyll-*a* precedes chlorophyll-*b* (64). Shlyck (65) showed the rate of chlorophyll metabolism in plants to be of the order of 5 per cent of renovated molecules per 24 hr. Turchin and collaborators (66) give a higher value, up to 30 per cent, in the experiments with labelled nitrogen. Kutyurin (67) gives the value of 8 per cent from the experiments with chlorophyll labelled with deuterium.

Gubbenet & Bazhanova (67a.) noticed changes of 20 to 30 per cent by measuring chlorophyll content in potato leaves in day time and at night.

In spectral measurements carried out by our group the rate of the accumulation and conversion of protochlorophyll in green bean leaves was evaluated (26). The results obtained are close to those of Shlyck *et al.* (63, 65). The biosynthesis replenishes chlorophyll partially destroyed during photosynthesis mainly through oxidation.

Sironval & Kandler (68), studying kinetics of chlorophyll bleaching in living *Chlorella* cells under high light intensity, showed the existence of an induction period which seems to be determined by the presence of protective substances. Carotene is the first to bleach, followed by chlorophyll-*a*, -*b*, and xanthophyll. Wolken & Mellon studied chlorophyll bleaching in *Euglena* chloroplasts in light and in the dark (69).

Thus, the data presented above allow one to perceive the following picture of the states of pigments in organisms.

Most bacterioviridin of green bacteria and bacteriochlorophyll of purple bacteria, is present in aggregated, regularly arranged forms of various types; it is this phenomenon which determines the peculiar absorption spectra of these pigments in living bacteria. Chlorophyll of green plants is present in aggregated and monomeric forms coupled and uncoupled with proteins and lipids. The equilibrium between different forms undergoes alterations in the process of chlorophyll formation and depends on the type of the organism and on physiological conditions. The nature of the bonds with proteins and lipids and that of the participation mechanism of different forms of pigments in photosynthesis has not been elucidated thus far. It may be that different forms of pigments participate in different stages of photochemical processes.

How can these ideas be coordinated with the lamellar structure of

chloroplasts as revealed by means of electron microscopy? The term "aggregation" refers not only to the interaction between the pigment molecules in "tridimensional" particles but also to the bidimensional arrangement of the pigment in monolayers. Different density of packing of chlorophyll molecules in different zones of grana may correspond to aggregated and monomeric forms; and last, a part of the pigment can probably be present beyond the lamellar structure.

Light Quantum Absorption and Luminescence

The process of photosynthesis begins from the absorption of a light quantum by plant pigments. The energy of quanta is utilized for the photochemical process, degrades to heat, and is emitted in the form of luminescence of various kinds. This phenomenon is of great importance for the comprehension of the nature of elementary processes following the absorption of a light quantum.

Singlet excited states.—It is generally assumed (70) that the chlorophyll absorption spectrum with two large absorption maxima in the visible region indicates the presence of two electronic excitation levels and of some vibrational sublevels.

Beside $\pi \rightarrow \pi$ transition of delocalised electrons of the system of conjugated double bonds under excitation, there was recently pointed out the possibility of $n \rightarrow \pi$ transitions from unshared electron pairs of nitrogen or oxygen atoms in the chlorophyll molecule. Franck (71) forwarded a hypothesis that both transition types are possible in the main chlorophyll absorption bands, whereas a different probability of triplet state formation exists in different transition types.

Gurinovich *et al.* (72), studying absorption spectra, polarization, and luminescence of chlorophyll and its derivatives reached the conclusion that characteristic electron transitions correspond to each absorption band. These results are in accordance with previous measurements (72a, 112). The study of luminescence polarization of chlorophyll and pheophytin in solution brought Kravtsov & Gruzinsky (73) to the conclusion that both maxima observed at about 680 and 730 mμ belong not to vibrational sublevels, but to different electron excitation levels.

Lifetime (τ) of excited singlet (S) states of chlorophyll and its derivatives were measured in three different laboratories (74 to 76), and the results obtained were in agreement. The τ value of excited chlorophyll in solution lies within the range of 5.10^{-9} to 8.10^{-9} sec., while in green leaves it is 2 to 3 times less. To interpret this phenomenon, a number of hypotheses were put forward: (*a*) only a part of chlorophyll in green leaves exists in the fluorescing form, and there is some outflow of energy to the nonfluorescing phase; (*b*) the value of τ decreases owing to high concentration of chlorophyll molecules in chloroplasts and to the energy exchange between the molecules (75).

Metastable (triplet) excited states.—In agreement with generally ac-

cepted ideas, an excited pigment molecule in the singlet state can, having lost some of its energy, transit to the metastable, long-lived excited state. The ideas on the participation of metastable chlorophyll molecules in the photochemical reactions of chlorophyll were put forward in the works of Franck & Livingston (77), who supposed the tautomeric nature of these states. In their independent works, Terenin (78) and Lewis & Kasha (79) based the contemporary, generally accepted idea on the triplet nature of metastable excited states of the molecules of aromatic organic compounds. Let us analyse the data on the possibility of the triplet (T) state formation of chlorophyll.

Delayed fluorescence.—Tollin *et al.* (80), studying the spectrum of delayed luminescence of chloroplasts at low temperatures, noted its resemblance to the fluorescence spectrum. This luminescence is probably emitted upon the transition $S^* \rightarrow S$ perhaps via $T \rightarrow S^* \rightarrow S$ because of a certain amount of heat energy at minus 150°C.

Phosphorescence.—Becker & Kasha (81) noticed chlorophyll phosphorescence in the near infrared, at low temperature. These data were confirmed by Terenin (82) with the use of a photoelectric method. No phosphorescence of chlorophyll-*a* in frozen solution could be asserted thus far.

Flash-spectroscopy.—A number of works showed that a transition of molecules to the triplet state takes place under the effect of a high energy flash of light. Thereafter, owing to the accumulation of triplet molecules, the main absorption band became weaker and the appearance of a new band corresponding to the transition $T \rightarrow T^*$ was observed. A suggestion was made by the present author (see below) that in such experiments spectra of photoreduced forms of chlorophyll were observed as a result of electron (hydrogen) acceptance from molecules of the solvent or admixture, considering the ready ability of cholorophyll to undergo a reversible photoreduction (83, 114).

All the experimental material available shows the probability of the formation of the triplet states of chlorophyll. However, the participation of these states in the process of photosynthesis requires further investigation.

Chemoluminescence.—Beside fluorescence and phosphorescence, due to the singlet and triplet excited states of chlorophyll molecules, new types of delayed luminescence of chlorophyll were found in organisms. Strehler & Arnold (84) discovered a weak afterglow in plants lasting some seconds after the light was turned off. This afterglow, by its spectrum, was close to chlorophyll fluorescence. The action spectrum of afterglow excitation showed its correspondance to that of photosynthesis (absorption spectrum of chlorophyll). This luminescence, which has to be considered as chemoluminescence, is sensitive to enzymic poisons and temperature and seems to be associated with the elementary processes of photosynthesis. Further study of this phenomenon lead Strehler *et al.* (85, 86) to the conclusion that this luminescence consisted of three components: a short one lasting 10^{-3} sec.; a long-lived one lasting minutes; and an intermediate one.

Enzymic toxins, such as hydroxylamine and dinitrophenol, depress the long-lived component in *Chlorella* cells; exposure to 50°C. does not affect the short-lived luminescence but inhibits the long-lived one which seems to be connected with biochemical events. In these works, correlation between luminescence and light absorption change by *Chlorella* cells was studied by the method of differential spectroscopy. Thereby a qualitative relation between the long-lived luminescence and the absorption change in leaves at 525 and 648 mμ was observed. Luminescence may be related to back reactions of photoproducts that possess absorption in the mentioned spectral region. Attention is drawn to the fact that the maximum at 525 mμ corresponds to the maximum of the photoreduced form of chlorophyll (see below). It should be noted that a weak chemoluminescence in back reactions (oxidation) of the photoreduced form of chlorophyll was found by our group (87). Its intensity greatly increased under the action of ammonia in accordance with its action on back reactions of photoreduced chlorophyll [see below and (129)].

Tollin *et al.* (88, 88a) studied luminescence of spinach chloroplasts at different temperatures. At room temperature, three kinds of afterglow with half-times of 0.15, 2, and 15 sec. were found. As the temperature dropped down to minus 35°C., only the short-lived luminescence component remained. At minus 90°C. a new type of luminescence with the half-time of 0.3 sec. is observed. This half-time increases with further cooling to minus 196°C.

Chloroplasts dried in a flow of nitrogen give, upon heating, two maxima of thermoluminescence at 50° and 150°C. in accordance with the work of Arnold & Sherwood (89). Freshly isolated moist chloroplasts did not show this effect. Calvin *et al.* (90, 91) point out a qualitative correlation of the observed luminescence of chloroplasts with results of measurement of electron spin resonance (ESR) of the same objects. Thus, both the luminescence and ESR are induced by the light absorbed by chlorophyll. The periods of time for extinction of luminescence and ESR are close at room temperature (lasting seconds). At minus 140°C. and in dry chloroplasts, ESR is preserved for hours and, in correspondence, no luminescence is found within the region of 700 to 900 mμ; at 60°C., ESR of dry chloroplasts lasts a few seconds only, and at this temperature a peak of thermoluminescence is also found. The luminescence observed is ascribed by the authors to the recombination of unpaired electrons and "holes" in the quasicrystalline lattice of chlorophyll in grana.

A different luminescence period is interpreted in this work by the different depths of holes trapping electrons mobilized by light. Low temperature luminescence may be ascribed either to the emission of a quantum from the lower triplet level, or to the transition to the excited singlet state at the expense of some reserve of heat energy.

It seems to us that the use of a semiconductor model simplifies the actual pattern of the process without taking into consideration its biochemical specificity. Instead of electron traps one may conceive the formation of

intermediate ion radicals of different lifetimes. Different types of luminescence may be interpreted by the recombination of free radicals having different lifetimes formed in the way of photochemical electron transfer (with the participation of chlorophyll) in the chain of intermediate enzymatic and redox systems.

Thermoluminescence.—Arnold & Sherwood (89) found luminescence arising upon heating dry chloroplast films after their preillumination with the visible light. A luminescence maximum was observed when exposing dry chloroplasts to 100 to 130°C. Luminescence was preserved during four cycles (illumination-heating-cooling), though the band became weaker with each subsequent cycle. The luminescence was found in the region of 630 to 800 mμ. Illumination at the liquid nitrogen temperature did not bring about any measurable luminescence effect after heating. Chloroplast conductivity was temperature dependent; upon heating illuminated chloroplasts, a minimum was observed at 60 to 70°C., while the cooled preparation showed a monotonous resistance decline upon heating. A conclusion is drawn by the authors that these phenomena resemble the well known thermoluminescence of crystalline semiconductors upon excitation with ultraviolet or x-rays and that therefore, the pattern characteristic of semiconductors is applicable to chloroplasts; in the process of light absorption, electrons move to the zone of conductivity and are trapped in the traps of various depths. Heating releases the electrons and brings about afterglows of different lifetimes.

In these experiments preparations were used similar to chlorophyll solid films, since the biological organization of the system and the native state of protein were destroyed upon heating chloroplast preparations up to 140°C.

It was necessary to clarify whether this phenomenon is characteristic of the peculiar state of chlorophyll or a common property of the solid films of chlorophyll and other pigments investigated by a number of workers (87).

In another paper (92), Arnold & Sherwood studied thermoluminescence of dry chloroplasts in relation to temperature. Analysis of the broad luminescence band made the authors conclude that there existed not only the possibility of electron trapping but also that of chemical compound formation. Of interest are the observations that this effect was obtained only in the presence of oxygen, while CO_2, H_2O, and N_2 were not necessary for the reaction. These observations show that the nature of these effects is probably connected with chlorophyll chemiluminescence, maybe under the effect of lipid peroxides formed as a result of chlorophyll sensitized photoxidation, rather than with the "physical" mechanism of thermoluminescence. This interpretation eliminates the necessity of the semiconductor model to explain this phenomenon (87).

Energy (electron) migration.—Much attention is given in the literature to possible mechanisms of the migration of energy of light quanta between chlorophyll molecules in grana and from the molecules of phycobilins and carotenoids to chlorophyll [cf. the reviews of Terenin (93, 93a) and Rabinowitch (94)].

The possibility of energy transfer between chlorophyll molecules in the chloroplasts in the singlet excited state is illustrated by the observations of Arnold & Meek (95) on fluorescence depolarization. Konev (96), by measuring phycoerythrin fluorescence polarization at room and low temperatures, showed that an energy exchange was likely to take place between many erythrobilin residues bound to protein in the chromoprotein molecule.

The phenomenon of energy transfer at the triplet level between different organic molecules was discovered by Terenin & Ermolaev (97); it was already pointed out that there existed a possibility of energy transfer in this way between different forms of chlorophyll in the chloroplasts (34). Franck (71) supposed the possibility of energy transfer in chloroplasts from chlorophyll molecules in the singlet state to those in the triplet state. In the case of a close interaction between chlorophyll molecules upon bi- or tridimensional aggregation in grana there is possible a migration of "the exiton" or in the extreme case, of electron and "hole," which leads to the appearance of photoconductivity.

Terenin & Putzeiko (98), Putzeiko (99), and Terenin (152) have actually observed photoconductivity of chlorophyll-*a* and chlorophyllids in the state of solid films. They have determined the "hole" type of photoconductivity and stated that the maxima of photoelectric sensitivity coincided with the absorption maxima of the crystalline chlorophyll.

Nelson (100) and Calvin (101) described experiments in which photoconductivity was observed in solid chlorophyll films, while Arnold & Maclay (102) noted photoconductivity of dry chloroplast films and of the films of chlorophyll and carotene. All these effects, however, are not specific to chlorophyll: in the experiments of Vartanyan (103) most organic dyestuffs were shown to possess photoconductivity in solid films. Besides, chlorophyll in chloroplasts is not in the crystalline state, which is shown by the differences in absorption maxima of chlorophyll in grana (678 mμ) when compared to crystalline forms (690 and 730 mμ). Photoconductivity at the level of the triplet state may be associated with the appearance of unpaired electrons upon illumination of chloroplasts; this fact is proved by the measurement of the electron spin resonance (ESR).

Commoner (104, 105) noted the appearance of an ESR signal upon illumination of chloroplasts and living cells of *Chlorella*. Calvin and coworkers (106) asserted that upon freezing chloroplasts and photosynthetic bacteria down to minus 150°C., illumination also led to the appearance of the ESR signal which disappeared after the light was switched off. An evaluation of the number of unpaired electrons was made, which amounted to from 1/100 to 1/500 of the total amount of chlorophyll molecules. A conclusion is drawn by the author that the appearance of the signal at minus 150°C. bears witness only to the shift of the electron, which is regarded as a photophysical process. The appearance of the ESR signal can be ascribed either to the formation of triplet states, or to the electron acceptance by the excited chlorophyll molecule, with the formation of a pair of

ion radicals. It is unclear, thus far, whether unpaired electrons measured by the ESR method are determined by photoconductivity or if these two phenomena are independent.

Thus, during recent years there were revealed all possible types of chlorophyll luminescence in chloroplasts, which are associated with the formation of excited states (fluorescence and phosphorescence) and with the reactions of primary photoproducts (chemoluminescence). The study of these phenomena produced important data on the primary acts of light energy conversion and on the pathways of electrons in photosynthesis, though the nature of the primary photoprocess is not convincingly proved in these experiments.

Primary Photochemical Process and "Storage" of Light Energy

Photochemistry of chlorophyll and its analogues.—In photosynthesis oxido-reduction takes place—the utilization of water hydrogen for CO_2 reduction. If chlorophyll is actually involved in the chain of reactions of hydrogen transfer from water to carbon dioxide, one has to look for the possibility of a reversible photochemical oxidation or reduction of this pigment accompanied by the "storage" of light energy in unstable, reversibly reacting, photoproducts of chlorophyll.

Reversible oxidation.—In 1937 Rabinowitch & Weiss (107) revealed the possibility of a reversible photochemical oxidation of chlorophyll by ferric ions. The present author showed the possibility of a partial reversible photooxidation of chlorophyll and bacteriochlorophyll by oxygen and benzoyl peroxide (108, 109). Dain & co-workers (110) studied the reversible chlorophyll photooxidation in its complexes with $FeCl_3$ and presented arguments in favor of the possibility of electron transfer in the complex with the formation of unstable chlorophyll oxidation products. It was suggested by the authors that the primary oxidation product with the absorption maximum at 510 mμ represented chlorophyll oxidized at the position 7–8 of its structure. Dilung (111) noted formation of unstable oxidation products upon the interaction between chlorophyll and oxygen.

Goedheer (112) confirmed our data on the reversible bacteriochlorophyll photooxidation with the partial regeneration of the pigment from oxidized products by ascorbic acid (109) and asserted the possibility of pigment oxidation by ferric ions and some other compounds.

Reversible photoreduction.—As early as in the end of the 19th century Timiriazev discovered the ability of chlorophyll reversible reduction by zinc.

The ability of chlorophyll to undergo reversible photochemical reduction accompanied by light energy storage in photoproducts thus formed was revealed in 1948 (113). Many works have been devoted since to the study of this reaction. It is now timely to review briefly these results [see also summarizing papers of the reviewer (114)].

Chlorophyll.—A short, red-light illumination of chlorophyll solutions in pyridine in the presence of a reductant (ascorbic acid or phenylhydrazine) under anaerobic conditions leads to the formation of a red photoreduction product with the absorption maximum at 523 mμ. The light being switched off, a back reaction occurs with the regeneration of chlorophyll, being accelerated by the admission of oxidants. As a side process, a partial pheophytinization was observed, in particular in the presence of an excess of ascorbic acid and upon prolonged illumination (113). The reaction could also be measured in alcohol in the presence of organic bases, pyridine, nicotine, phenylhydrazine, and hexamethylen-tetramine, and in pyridine in the presence of up to 50 per cent of water (115).

Continuing the study of photoreduction in our laboratory, Evstigneev & Gavrilova (116) noticed that chlorophyll solution in toluene is also photoreduced by phenylhydrazine. Under these conditions there was asserted the possibility of photochemical chlorophyll-*b* reduction with a good reversibility. At a temperature lowered down to minus 40°C. the appearance of the red photoreduced form of chlorophyll was extremely slow.

Holt & Rabinowitch (117) noticed that the "photochemical reduction—back reaction" cycle could be repeated many times with an ever increasing amount of the irreversibly changed chlorophyll. Linschitz & Weissman (118) observed the appearance of the red photoreduced form of chlorophyll in phenylhydrazine. Lynch & French (119) reproduced photochemical chlorophyll reduction in pyridine and pointed out that this reaction could be observed upon addition of 50 per cent pyridine to the aqueous chloroplast suspension. Rackow & König (120), studying photochemical chlorophyll reduction, showed that upon cleavage of the cyclopentanone ring of the chlorophyll molecule they failed to notice photoreduction, a fact which, in their opinion, proved that this group of the molecule was indispensable for the reaction to occur.

This conclusion seems to be an erroneous one, since a number of other chlorophyll analogues lacking the cyclopentanone ring (such as porphyrins, magnesium phthalocyanin) are completely able to undergo photochemical reduction.

When studying the reaction of reversible photochemical chlorophyll reduction, Bannister (121) reached the conclusion that the reaction occurred only upon the addition of either alcohol or water to pyridine, which promoted the formation of ascorbate ion, an active electron donor. The quantum yield of the formation of the "red" reduced form attained 0.05 at 40 per cent of water in pyridine. Pheophytinization is observed during the reaction. In ether solutions, freed of oxygen, chlorophyll is photochemically reduced by phenylhydrazine; the reduced form has maxima at 410 and 520 mμ.

In accordance with the results of our laboratory, Bannister suggested participation in the reaction of the triplet but not of the singlet excited state of chlorophyll.

Pheophytin.—We noticed a reversible photochemical pheophytin reduction (115) with the appearance of the "red" form resembling in its spectrum the corresponding form of chlorophyll. Comparative studies of chlorophyll and pheophytin were carried out in the work of Evstigneev & Gavrilova who found a more rapid pheophytin reduction. In pyridine solution at minus 40°C., a brown coloured intermediate is formed, which upon heating undergoes transformation to the "red form" (122). A detailed study of this primary reduced form of pheophytin and other pigments was made by Evstigneev (123).

Ethylchlorophyllid.—This substance behaves similarly to chlorophyll, forming the "red" reduced form. A reversible photochemical chlorophyllid reduction in the presence of versene was noted by Holt (124).

Protochlorophyll.—When protochlorophyll is photochemically reduced by ascorbic acid it forms a brown photoproduct with the absorption maximum at 470 mμ and reversibly reacts back (oxidized) to form the green protochlorophyll (125). However no chlorophyll formation could be observed, though prolonged illumination gave a small amount of a product with the maximum at 675 mμ. Protopheophytin is also able to undergo reversible photochemical reduction.

At minus 40°C., absorption spectra were measured of the primary photochemically reduced form of protopheophytin which, by its properties, was analogous to the primary form of pheophytin (126, 126a).

Bacteriochlorophyll.—Photochemical reduction by ascorbic acid in pyridine leads to the formation of photoproducts lacking characteristic absorption spectra in the visible (109). The reaction goes further and is more readily reversible in more basic media as in the presence of piperidine, ammonium, or phenylhydrazine as the reductant. With sodium sulfide, the reaction is rapidly reversible. Under these conditions bacteriochlorophyll and bacteriopheophytin form a green product of photoreduction (109).

Bacterioviridin (127).—There was observed the "red" photochemically reduced form resembling the product of chlorophyll-*a* photoreduction. Pheophytin obtained from bacterioviridin also resembles, by its properties, the pheophytin-*a*, giving the primary and secondary photoreduced forms. Bacterioviridin differs from chlorophyll in a higher rate of photooxidation in solution, which brings it closer to bacteriochlorophyll in its behavior.

Porphyrins.—Behaviour of protoporphyrin, hematoporphyrin, porphyrin of photosynthetic bacteria, and the porphyrin obtained from protochlorophyll turned out to be similar; differences were found only in the rate of photo- and back reactions (128 to 130).

Our group studied photoreduction of these compounds in organic solvents, in aqueous acid and alkaline solutions. In the bases, piperidine and pyridine, the reaction with ascorbic acid usually gives a green photoproduct with the absorption maximum at 640 to 660 mμ which, on admission of air, undergoes reversible conversion to porphyrin. In water, in the presence of 10 per cent pyridine, a photoproduct is formed with an absorption maxi-

mum at 740 to 750 mμ (128). Both compounds are determined by the conditions of the acid-basic equilibrium (129).

In aqueous acid solutions the absorption spectra of porphyrins undergo marked alterations owing to the addition of protons by nitrogen atoms of pyrrol nuclei, and an intermediate photoreduced product with the absorption maximum at 520 mμ is formed (130). At minus 40°C. there was observed the appearance of the "primary" form of hematoporphyrin and protoporphyrin with the maximum at 450 mμ; upon heating, this form partially transits to the "green" photoreduced form with an absorption maximum at 650 mμ (126).

Complexes of chlorophyll with metals.—A detailed study of the photoreduction of chlorophyll ferric ion complexes was undertaken by the Dain group (131, 132); they suggested that in the reaction there takes place electron transfer in the complex of Fe-chlorophyll with OH ions, water molecules, and metal ions which serve as a reductant. Dain & Ashkinazi (132) made a suggestion that in the excited state the electronic system of these pigments is unstable. This instability leads to the subsequent electron transfer after the act of a light quantum absorption within the complex from the organic skeleton of the molecule to the central ferric ion, which is thereby reduced. The electron deficiency thus formed is replenished at the expense of the solvent molecule oxidation. As the act of electron transfer immediately follows the act of light absorption, a significant reduction of the lifetime of the excited state takes place. It is manifested in a certain broadening of absorption bands, in the disappearance of vibrational structure and fluorescence (132). No photoreduction could be noticed of cupric-complexes of pheophytin, but a fluorescing zinc complex was able to undergo photoreduction. In the experiments of Vostrilova & Dulova (132a) and Rackow & König (120) a good reversibility of the photoreduction of this compound was observed. A very slow photoreduction of cytochrome-*c* under the action of the visible light was observed by our group (133, 133a).

Phthalocyanins.—Upon illumination of magnesium phthalocyanin solution in pyridine with ascorbic acid, along with the formation of irreversibly reduced products (113), there was noticed a process rapidly reversible in the dark (134). Carrying out this reaction at low temperature, Evstigneev & Gavrilova (135) found a rapid formation of the "red" photoreduced form with the maximum at 550 mμ, which quickly underwent a back reaction upon the admission of air. Just as in the case of chlorophyll, they were unable to measure the spectrum of the extremely unstable "primary" photoreduced form of this compound.

Carotenoids.—An attempt was made to carry out photoreduction of carotene and xanthophyll in different media with different reductants but no formation of reversibly reacting products of these compounds could be noticed (136).

Phycobilins.—Our experiments showed phycoerythrin and phycocyanin

to be lacking both the ability to undergo this reaction, and the photosensitizing action in solutions (137). Attempts were made to photoreduce erythrobilin obtained under acidic hydrolysis of phycoerythrin, but this compound was also unable to undergo this reaction. Experiments on bilirubin also gave negative results (126).

The role of the pigment structure.—The reaction of photoreduction could be noticed with all derivatives and analogues of chlorophyll possessing the structure of chlorin, bacteriochlorin and porphin with central atoms of hydrogen, magnesium, or zinc. Different pigments differ in spectral properties and in the reactivity of their reduced forms in different media. Magnesium complexes usually form more active (rapidly back-reacting) reduced forms than pheophytins. The main feature determining the ability of pigments to undergo reversible photoreduction is the presence of a circularly conjugated system of double bonds embracing four pyrrol rings of pigment molecules.

Change of the redox potential (E_o) in photoreaction.—If the chlorophyll-ascorbic acid system in pyridine was able to reduce compounds with E_o up to 0.05 (methylene blue) prior to illumination, a reduction of compounds with E_o up to minus 0.35 (safranin T, pyridine-nucleotides) was noticed after illumination (138). These data received confirmation by measurement of the potential of the inert electrode in photoreacting systems. A sharp potential change of such an electrode was observed upon illumination of the chlorophyll-ascorbic acid system (138), similar to that of the Rabinowitch experiments (139) for the thionine-ferrous ion system.

The photopotential of a platinum electrode was studied in detail by Evstigneev & Gavrilova, following illumination of the reacting solution (140). It was shown that the photopotential was determined not by the illumination of the electrode itself but by the formation of photoproducts in the solution. Upon lowering the temperature, an increase of the E_o value was noticed at the expense of diminishing the back-reaction rate and increasing the stationary concentration of the photoproduct. The change of potential was followed at minus 40°C. (i.e., under conditions when no "red" form of chlorophyll was formed); these observations led Evstigneev to the conclusion that the potential was determined by the formation of the primary "electrode-active" reduced form of pigment. This conclusion was confirmed in the work of Bannister (121) who investigated the quantum yield of the reaction of chlorophyll photoreduction in relation to reductant concentration.

Upon illumination of the solutions of chlorophyll and its analogues with ascorbic acid, the value of E_o attained minus 0.35 v. which is in agreement with the experiments with the use of redox indicators (138). Thus the "electrode-active" and the "red" reduced forms have similar E_o values.

Hendrich (141) noted a change of photopotential upon illumination of the chlorophyll-ascorbic acid system and studied the action of a number of

inorganic ions on this process. He noted that Cu^{++} and Ni^{++} ions which were able to react with ascorbic acid prevented the appearance of photopotential.

When illuminating a chlorophyll-phenylhydrazine system in vacuum, Evstigneev & Gavrilova found an increase of photoconductivity (142). With the light turned off, the electroconductivity returned to its previous level, thus bearing witness to the formation of photoproducts of ionic nature. Comparison of the kinetics of photoconductivity, appearance of photopotential, and spectral data on pheophytins at various temperatures, show that just the primary electrode-active products determine the appearance of photoconductivity. The shift of platinum electrode potential after illumination of the chlorophyll-ascorbic acid system in pyridine to the negative side bears witness to the formation of electrode-active products (photoreduced form of pigments) that give up their electron to the electrode in accord with the scheme of the primary photoprocess: $Chl + AH \rightleftarrows \cdot Chl^- + \cdot AH^+$.

Thereby a question arises why a platinum electrode, becoming negatively charged, accepts the charge of the reduced form of the pigment but does not give up the electron to the oxidized form of the hydrogen donor.

It would be interesting to compare this phenomenon in the case of a reductant of some other chemical nature. The photopotential of a platinum electrode in acidic aqueous solutions in the porphyrin-ascorbic acid system was taken under study (130); in this system the photopotential shifted to the negative side, just as the case of the chlorophyll-ascorbate system in pyridine. However, on application of ferrous oxalate as an electron donor, the photopotential shifted to the positive side, as in this case ferric ions (probably formed in the elementary photoprocess; $Chl + Fe^{++} \rightleftarrows \cdot Chl^- + Fe^{+++}$) were apparently more electrode active. Kinetics of the changes of electrode photopotentials in the systems under study require further investigation.

Reductants.—Reaction in its most pronounced form was observed with pyridine as the medium, in the presence of ascorbic acid and other dienols, phenylhydrazine, hydrogen sulfide, and cysteine (114). To analyse a rapidly reversible, "latent" formation of primary photoproducts, methods should be applied that permit following up the formation of these compounds during the period of illumination. Most sensitive in this case were methods of photopotential measurement, sensitized photopolymerization, or sensitized reduction of irreversibly reduced electron acceptors.

In Evstigneev's experiments, an E_o change to the negative side on reaction with chlorophyll was shown by hydroquinone, pyrocatechine, thiosinamine, and a number of other compounds (123). Oster (143) used ethylenediamine tetracetic acid in the reactions of this type. The polymerization method (144, 144a) also showed activity of ferrous compounds. Sensitized cytochrome oxidation in the presence of chlorophyll (133) suggests the possibility of a reversible process in this case as well. Thus electron acceptance by excited chlorophyll molecules is possible with the

participation of systems possessing an E_o from minus 0.3v. up to plus 0.3v. However, the possibility of electron acceptance by the excited pigment molecule from a water molecule remains, as yet, uncertain.

Deuterated electron donors.—Application of deuterated ascorbic acid leads to the retardation of chlorophyll photoreduction with the formation of the "red" form; the back, dark reaction also runs more slowly (145). The Hill reaction is also slowed down upon the increase of D_2O amount in the water medium (145). Thereby, D_2O, slowing down the formation of the "red" form of pheophytin, does not affect the formation rate of the "primary" photoreduced form. At low temperature the formation rate of the primary form of pheophytin is the same with the use of both deuterated and nondeuterated ascorbic acid (146). These experiments also prove the electron transfer during the formation of the "primary" reduced form and proton participation in the formation of the more stable "red" form.

Reaction with the solvent.—Porret & Rabinowitch (147) were the first to observe the effect of reversible chlorophyll bleaching in methanol. Livingston (148, 148a, 148b) studied reversible spectral changes of chlorophyll solutions by means of flash spectroscopy and found an absorption increase at 525 mμ upon illumination, which corresponded to the maximum of the photoreduced form of chlorophyll. Linschitz & Rennert (149) noticed reversible changes of the chlorophyll absorption spectrum upon its illumination in vitreous media at low temperature.

Applying the technique of flash photolysis, Linschitz & Abrahamson (150) found in pyridine solutions of chlorophyll a reversible decrease of the chlorophyll absorption maximum in the red and the appearance of a maximum in the green at 525 mμ, as well as a certain absorption increase at 700 mμ. The authors note that the lifetime of the product with the absorption at 525 mμ was greater in pyridine than in other solvents and the reversibility of the process in pyridine was better than in purified methanol.

By means of the flash photolysis technique, Terenin and co-workers (151) found a reversible decrease of the absorption maximum in the solutions of magnesium phthalocyanin, as well as the appearance of new maxima in haematoporphyrin (152).

A suggestion was made earlier by the present author (83, 114) that effects observed in the described flash spectroscopy experiments were due to the reversible chlorophyll photoreduction when the solvent molecules served as electron (hydrogen) donors. Reversible effects in this kind of experiments were actually inhibited by oxygen, which was in agreement with the rapid reaction of oxidants with the photoreduced form of chlorophyll.

In the experiments of Livingston & Fujimori (153) the action of various compounds on τ-lifetime of metastable excited states of chlorophyll was studied. Oxidant molecules were found to reduce the τ value, while reductants (ascorbic acid) did not affect it. These results are understandable when one assumes that a photoreduced form of chlorophyll is formed by

light flashes. Naturally, this form does not react with the reductant (ascorbic acid), but it does react with the oxidant (quinone). Carotene action in these experiments corresponds to its effect on the photoreduction of chlorophyll revealed in our experiments (154).

The spectrum of the "triplet" state in the papers of Livingston & Linschitz resembles that of the "primary" photoreduced form of pheophytin measured by Evstigneev (123, 126). It also has the absorption region corresponding to the "red" form of chlorophyll. The effect of the electron (hydrogen) acceptance is apparently the main feature of these experiments, while the possible intermediate formation of the triplet state, with shorter lifetime, overlaps the main effect, thus being hardly distinguishable by the registration technique applied. [See also 152].

Acid-base properties of the medium.—A distinct formation of relatively stable photoreduced forms of pigments may be noticed in pyridine. This stability seems to be related to the conditions of the acid-base equilibrium, when a proton is situated "between" the pigment molecule, which has accepted an electron, and the molecule of the base (the medium).

$$\cdot X^- \ldots\ldots H^+ \ldots\ldots N\lessdot$$

Formation of these relatively stable compounds is possible only if certain values for the affinity of the pigment molecule to the proton and the medium (the base) are kept. The optimum of the basicity at which stable reduced forms are formed, varies in different pigments. It should be expected that in a medium of higher basicity than pyridine, the formation of negative ion radicals $\cdot Chl^-$ rapidly reacting back, will dominate. In more acidic media one might expect a formation of radicals $\cdot ChlH$ upon the acceptance of one proton, and a formation of positively charged ion-radicals $\cdot ChlH_2^+$ upon the acceptance of two protons.

When adding some drops of a strong base, such as piperidine or ammonium, to the solution of the "red" reduced form of chlorophyll in pyridine, a rapid back-reaction occurs (at the expense of the reduced form ionization). Corresponding to this, piperidine or ammonium, added to chlorophyll solution in pyridine inhibit the formation of the "red" reduced form in photoreactions (129). If the reaction is run in pyridine carefully dried with calcium hydride then the formation of the "red" form proceeds much more slowly than in the case of usual reactive moist pyridine, due to a deficiency of "hydrogen ions" in the dry pyridine, which are necessary for the formation of the "red" form.

In the case of hematoporphyrin, in strongly basic media (piperidine), a reduced form with the absorption maximum at 650 mμ appears while in more "acid" media (alcohol-pyridine mixtures) the one with the absorption maximum at 740 mμ is formed. By altering the basicity of the medium one can repeatedly and reversibly change the position of the absorption maximum of reduced forms (129).

It should be pointed out that when reducing pigments by zinc, by

Timiriazev's method, an acidity optimum is observed for the formation of reduced forms of various pigments (155).

Formation of free radicals.—Molecules serving as oxidants (quinones, nitro-compounds) quench chlorophyll fluorescence but do not react with it photochemically, while the reductants, reacting, do not cause "physical" quenching of pigment fluorescence (156). These observations show the possibility for the participation in the reaction of just the metastable (triplet) pigment molecules. The reversal of an electron spin during the formation of the triplet state determines the long life of the "hole," i.e., of the vacant electron level.

In the elementary act of photoreduction electron acceptance takes place by the excited chlorophyll molecule; therefore formation of an ion-radical pair must take place. The subsequent act of proton transfer may lead to the formation of a radical pair in the non-ionic form.

$$\mathrm{Chl} + \mathrm{AH} \rightarrow \cdot\,\mathrm{Chl}^- + \cdot\,\mathrm{AH}^+ \rightarrow \cdot\,\mathrm{ChlH} + \cdot\,\mathrm{A}$$

Uri (157) noticed that chlorophyll induces methyl-metacrilate polymerization in the presence of ascorbic acid. A detailed study of sensitized photopolymerization by the pigment-hydrogen donor system in pyridine solutions and aqueous emulsions was undertaken in our laboratory (144, 144a). Polymerization is induced by chlorophyll, and all its analogues and derivatives. Phycobilins, in accordance with their inability for photoreduction, do not affect polymerization. Dienols, cysteine, and ferrous compounds proved to be the most active as electron donors. Reaction was inhibited by those compounds (safranin, riboflavin) that easily react with photoreduced forms of pigments.

Under anaerobic conditions, polymerization appears to be induced by ion radicals—primary reduced forms of pigments—while in the presence of oxygen it is induced by peroxide radicals, the products of the oxygen sensitized photoreduction (OH, HO_2). As to the Rosenberg comment (158) concerning the possible action of the triplet state of pigments in these experiments, it should be noted that active polymerization was observed only in the presence of molecules that served as electron donors. Linschitz & Weissman (118) were unable to find paramagnetic resonance absorption in the "red" reduced form of chlorophyll, and it is, as yet, unclear whether this form is a radical or a product of dismutation, $ChlH_2$. The second possibility should be regarded as a more likely one. Illumination of chlorophyll-ascorbic acid and pheophytin-ascorbic acid systems in vacuum, directly in a resonator, led in our experiments (144a) to the appearance of the ESR signal, which disappeared instantaneously after the light was turned off. No effect could be observed without a reductant.

Commoner (159) noted at the 4th International Congress of Biochemistry held in Vienna in 1958 that the structure of the ESR signal observed in chlorophyll photoreduction corresponded to the structure found after ascorbic acid oxidation and it appeared to belong to monodehydroascorbic

acid. This compound probably had a greater lifetime than the radical of chlorophyll. These conclusions are in agreement with the work of Bubnov *et al.* (159a) who measured ESR by photoreduction of chlorophyll and other pigments, and in green leaves.

Mechanism of photoreduction.—Using the ideas of Michaelis on the "one-electron mechanism" of oxido-reductive reactions, the following scheme of the reaction was suggested (113).

(*a*) Electron acceptance by the excited (probably, triplet) pigment molecule (Chl) from the hydrogen donor molecule (AH_2) with the formation of an ion-radical pair. We have pointed out the possibility of electron acceptance by the system of circularly conjugated double bonds of the pigment molecule.

$$Chl + AH_2 \rightleftarrows \cdot Chl^- + \cdot AH_2^+$$

(*b*) Transfer of the proton (linked to the molecules of the base-medium) with the formation of a radical pair and subsequent formation of compounds with saturated valences as a result of dismutation:

$$\cdot Chl + \cdot AH_2^+ \rightarrow \cdot ChlH + \cdot AH \rightarrow ChlH_2 + A$$

Experimental material obtained in our laboratory confirmed this general scheme of the process.

Thus, to sum up this section; under the effect of light, reversible oxido-reductive transformations of chlorophyll occur, whereby the reversible photoreduction is accompanied by a "storage" of light energy in photoproducts. This conclusion justifies looking for these types of reactions in living organisms.

Photochemical Pigment Transformations in Organisms

The study of isolated pigments revealed their reversible photochemical changes; the use of suitable media and electron donors and acceptors permitted the accumulation of products storing light energy and facilitated the study of their properties. Revelation of photochemical pigment conversions directly in organisms is a much more difficult task, since pigments occur in different states and their transformations are coupled with the chain of subsequent enzymic processes, so that one can hardly expect to find any considerable accumulation of transformed pigment forms under normal physiological conditions.

Measurements of fluorescence and light absorption are particularly suitable for studying rapid processes in living intact organisms.

Some twenty years ago, in the experiments carried out in the laboratories of Kautsky, Wassink, and Franck, fluorescence of leaves after the periods of light and darkness was studied under different conditions. The results were usually interpreted in physical terms. We have already mentioned that these changes of fluorescence upon illumination were probably related to the change of the state and photostationary concentration of the fluorescent

form of chlorophyll (34, 38). Thus the usual intensity decline of fluorescence upon illumination and its reappearance after a dark period had to be explained by the accumulation of photochemically changed (photoreduced?) forms of chlorophyll and by their reversible regeneration. Analogous fluorescence changes are observed, for example, upon chlorophyll photoreduction in model systems.

Our group (26) was able to show reversible changes in the chlorophyll fluorescence spectrum of leaves upon illumination not only at 20°C. but at the liquid nitrogen temperature as well, apparently at the expense of the primary photochemical process.

In recent years, with the improvement of spectroscopic technique, there was opened the possibility of studying small and rapid changes of optical density (ΔD) taking place in living organisms upon illumination.

Differential spectra that demonstrate the difference in absorption spectra between an illuminated and non-illuminated object are characterized by a number of maxima indicating the increase (positive maxima) or decline (negative maxima) of the optical density in different spectral regions. Analysis of these spectra and their comparison with differential spectra of known compounds (e.g., of the oxidized or reduced cytochrome) allow their interpretation in certain cases.

One should consider therefore that the change of the optical density in maxima seen in these experiments is usually of the order of some tenths of a per cent, so that the possibility of change of selective light scattering, as well as that of absorption can not be ruled out [Latimer & Rabinowitch (160), and Latimer (161)]. Interpretation of the results obtained is contradictory. Some authors ascribe the differential absorption spectra obtained by them to the change of cytochromes, flavins, carotenoids and other pigments present in plants. We shall be considering here only the possibility of chlorophyll transformations.

Duysens (162) was the first to obtain a positive maximum at 515 to 520 mμ after illumination of *Chlorella;* these results received confirmation in the works of Witt (163), Bell (164), Strehler & Lynch (85), and others. Attention was drawn to the fact that this maximum corresponded to the photoreduced form of chlorophyll (133). In all these works, however, measurements were carried out in the region of 350 to 600 mμ. To solve the problem of the chlorophyll changes and their nature, the range of measurements had to be extended to the red spectral region. This was actually done by Coleman, Holt & Rabinowitch (165, 166). They showed that a negative chlorophyll maximum at 680 mμ corresponded to the positive one at 520 mμ.

Kok (167) when investigating a number of organisms, found, along with the maxima in the red region, a new negative band in the region of 700 to 705 mμ, which was ascribed to a hitherto unknown pigment. Comparing differential spectra after illuminating *Chlorella* and after the reaction of chlorophyll photoreduction, Coleman & Rabinowitch (166) pointed out their similarity. A richer set of maxima in *Chlorella* shows the possibility

of simultaneously ocurring changes of cytochromes, carotenoids, and flavins.

However, the fact must be explained that upon chlorophyll photoreduction the ratio of $\Delta D_{670m\mu}$ to $\Delta D_{520m\mu}$ usually is 2 to 3, while in *Chlorella* cells it hardly reaches unity.

Witt *et al.* (168) noted that the absorption decline at 430 mμ, in the blue absorption maximum of chlorophyll, corresponded to an increase of the maximum at 515 mμ. The red chlorophyll fluorescence simultaneously undergoes reversible changes.

Of importance is kinetic measurement of these transformations. Different authors pointed out the presence of both rapid and slow stages of ΔD changes in different maxima (Witt, Bell, and Strehler). In works of our laboratory slow changes of D (lasting seconds) in the red chlorophyll maxima were observed in homogenates of sugar beet leaves (169).

Study of the kinetics of ΔD changes at 515 mμ showed the process to be composed of a rapid stage (the order of 10^{-5} sec.), temperature independent, and a slow one (10^{-2} sec.), temperature dependent, and associated with the dark stage of the process [Witt *et al.* (163, 170, 170a)]. Different stages of the process, as in the case of photosynthetic luminescence, may be possibly connected with different types of photochemical processes: with rapid ones of electron transfer and slower ones of hydrogen (proton) transfer and dismutation of primary formed radicals.

Bell (164, 171, 171a) made a suggestion that the ΔD change at 515 mμ was an accessory effect of photosynthesis, on the basis of the following observations: the presence of the effect in air devoid of CO_2, and in a leaf in which photosynthesis was suppressed by phenylurethane. The effect was saturated at light intensities much lower than those required for the saturation of photosynthesis. A sharp decrease of the value of the effect under the conditions close to anaerobiosis and under the continued action of exciting light during 5 to 10 min. only was observed.

Differential spectra measurements were carried out on photosynthetic bacteria also. Duysens (9, 172) noticed reversible absorption changes of bacteriochlorophyll maxima in purple bacteria. Goedheer (10) found marked bacteriochlorophyll changes in bacteria chromatophores in the presence of ferric ions and associated these changes with bacteriochlorophyll photooxidation. Similar results were obtained in the work of Smith & Ramirez (173).

The above described experiments show the onset of the development of a very important region of investigations: pigment transformations in living organisms. A definite conclusion can hardly be made at this time, but the experiments show rather definitely the possibility of reversible photochemical chlorophyll changes.

Further investigation must reveal whether these changes are related to chlorophyll participation in the process of photosynthesis.

Joint action of two light quanta during photosynthesis.—In recent years

data appeared showing that simultaneous action of light of different wavelengths could favour the course of photosynthesis.

Warburg *et al.* (174) noticed that the action of blue-green light of a very low intensity (maximum of the action spectrum at 460 mμ) greatly increased the quantum yield of *Chlorella* photosynthesis under the action of red light. Emerson (175) stated that upon the action of blue light, the long wave limit of *Chlorella* photosynthesis shifted to the extreme red region. Myers & French (176) measuring the action spectrum of the Emerson effect showed its similarity to the absorption spectrum of chlorophyll-*b*. A suggestion was made that blue light could also activate photocatalytic systems different from chlorophyll.

Voskresenskaya (177) noticed that in the region of 450 to 580 mμ oxygen uptake was increased in a number of plant organs containing no chlorophyll; the activity of flavin and cytochrome systems was shown. We found some acceleration of cytochrome-*c* reduction and oxidation upon illumination (133).

Light can also affect the activity of intermediate (photoreduced) forms of chlorophyll and its analogues, accelerating back reactions. There are some experimental data in this respect (178). We have already mentioned the hypothesis of Franck (71) on the joint action of excited chlorophyll molecules in the singlet and triplet states.

It should be said that the joint action of red and blue quanta seems to be not indispensable for photosynthesis, as the plants may apparently grow in the red light only.

Photosensitizing Action of Chlorophyll in the Processes of Hydrogen (Electron) Transfer

This type of reaction was studied in systems of different organization, such as pigment solutions, leaf homogenates, and chloroplast suspensions. These reactions may be regarded as separate links—"fragments"—of the photosynthetic chain, and therefore the elucidation of their mechanisms will contribute to the understanding of the course of the entire process occurring in living organisms.

Reactions in model systems.—Long ago in the experiments carried out in the laboratories of Baur, Gaffron, and others, chlorophyll in solution was shown to be able to sensitize oxidation of some organic compounds by oxygen and certain azo-dyes.

In our experiments upon illumination of the system chlorophyll-electron donor—electron acceptor with the red light we noticed a sensitized reduction of the electron acceptors at the expense of light quanta absorbed by chlorophyll (179 to 181). All chlorophyll analogues studied that were capable of photoreduction were found to possess this sensitizing action. In the case of bacteriochlorophyll and bacteriopheophytin, reactions were excited in the near infrared (182).

With ascorbic acid as the electron donor, we found photoreduction of

safranin, riboflavin (179), and pyridine-nucleotides (180, 181). The light being switched off, these reactions go back at different rates, sometimes requiring oxygen to regenerate the dye from the leucoform.

In these reactions, light energy "storage," i.e., an increase of the free energy of the system, takes place and is characterized by the value of the difference between redox potentials of the hydrogen donor (ascorbic acid $E_o = 0.05$ v.) and hydrogen acceptor (pyridine nucleotides $E_o = -0.32$ v.).

In aqueous medium, chlorophyll absorbed by cytochrome-*c* sensitizes oxidation-reduction of this compound (133). In cytochrome photoreduction it is probably the proteins that serve as electron donors. Photosensitized oxidation of cytochrome was observed in the presence of air (133). With the use of azo-dyes as electron acceptors, their irreversible reduction takes place. The azo-dye, methyl red, was long ago applied in this type of reaction (e.g., 183). In some sensitized reactions that are irreversible under aerobic conditions, oxygen is the final electron acceptor.

Photosensitized oxidation of a number of hydrogen donors that were able to photoreduce chlorophyll and pheophytin, and photosensitized oxidation of reduced forms of cytochrome-*c* and pyridine-nucleotides were noticed by our group (181, 182, 184, 184a). It is most likely that in this case the dark stage of the reaction comes to chlorophyll regeneration from the intermediate photoreduced form at the expense of its oxidation by oxygen. An alternative mechanism of the primary photoformation of chlorophyll peroxide with the dark act of its reduction by a hydrogen donor is less likely, though labile products of the photooxidation of chlorophyll and bacteriochlorophyll interacting (slowly) with ascorbic acid to regenerate the pigment were found (108, 109); the reaction of chlorophyll photoreduction by hydrogen donors proceeds much faster than the photoreaction with oxygen. High quantum yield of sensitized photooxidation seems to be determined by very rapidly occurring irreversible oxidation of photoreduced forms of chlorophyll by oxygen.

The fact that the sensitized reactions studied do actually pass the stage of the reversible photoreduction of chlorophyll is confirmed by the following observations:

(*a*) Sensitized reduction of the hydrogen acceptors is noticed only in a system which contains hydrogen donors capable of participation in the reaction of chlorophyll photoreduction.

(*b*) In the three-component system containing electron donor, pigment, and electron acceptor no accumulation of photoreduced forms of pigments is found due to their rapid reaction with the electron acceptors. The whole acceptor molecules having been "sensitizingly" reduced, only then the formation of a photoreduced form of the pigment sensitizer takes place. This is displayed in the form of the induction period in kinetic studies of the sensitized reaction (130).

(*c*) At last, it is possible to carry out separately the photostage of the

reaction (formation of photoreduced forms of the pigment) and the reaction in the dark with the electron acceptors (138). In the case of chlorophyll this is illustrated by the experiments on the "red" photoreduced form; the "primary" photoreduced forms, however, react much faster with the acceptors, as was shown in the experiments of Evstigneev. The action of acceptors on the electrode photopotential in the chlorophyll-ascorbic acid system is significant (140), as is the "quenching" action of these compounds on the photoconductivity of the chlorophyll-ascorbic acid system (185) owing to their rapid reaction with the primary photoreduced form of chlorophyll. The mechanism of these reactions is the following:

(*a*) Photoreduction:

$$\mathrm{Chl} + \mathrm{AH_2} \rightleftarrows \cdot\,\mathrm{Chl^-} + \cdot\,\mathrm{AH_2^+} \rightleftarrows \cdot\,\mathrm{ChlH} + \cdot\,\mathrm{AH}$$

(*b*) Dark reactions of the photoreduced form with the hydrogen (electron) acceptor B; chlorophyll regeneration:

$$\cdot\,\mathrm{Chl^-} + \mathrm{B} \rightarrow \mathrm{Chl} + \cdot\,\mathrm{B^-}$$

$$\cdot\,\mathrm{ChlH} + \mathrm{B} \rightarrow \mathrm{Chl} + \cdot\,\mathrm{BH}$$

(*c*) Dismutation of semiquinone forms:

$$\cdot\,\mathrm{B^-} + \mathrm{H^+} \rightarrow \cdot\,\mathrm{BH};\ 2\mathrm{BH} \rightarrow \mathrm{B} + \mathrm{BH_2}$$

This mechanism of chlorophyll sensitization experimentally shown in our laboratory is similar to the Weiss scheme (186) proposed for the dyes-sensitizers that are able to form leuco-compounds.

Such a reaction mechanism is proved by direct experiments in solutions. However, in the case of heterogenous sensitization on pigment crystals and in colloidal solutions it is very difficult to separate individual stages of the reactions occurring at the phase boundary. One may think that the elementary acts of the electron transfer from the acceptor to the pigment molecule ("hole") at the phase boundary and the compensating act of giving back the electron to the acceptor molecule are retained here too. The possibility of such a mechanism was suggested in the experiments studying photocatalytic action of heterogenous sensitizers (187).

This possibility is also shown by the experiments of Evstigneev & Terenin (188) on measuring photopotentials of solid films of chlorophyll and its analogues in electrolyte solutions and by the recent experiments on chlorophyll sensitization in heterogenous systems (189).

Thus, "simple" reactions of hydrogen transfer occur with chlorophyll intermediate photoreduction in model systems; probably, reactions of this type are a link of photobiological processes in leaf homogenates and chloroplast suspensions where the primary photoproducts are metabolized with the participation of enzymic systems.

Involvement of Photoproducts in the System of Biochemical Processes

Chloroplast enzymes.—It was known long ago that a combination of photochemical and dark biochemical enzymic stages took place in photosynthesis.

One of the ways leading to the elucidation of these stages is the study of enzymic activity of pigment-bearing structures, i.e., of plant chloroplasts and bacterial chromatophores. This problem was given a close attention in recent years.

A comprehensive review of this problem was recently given by Sissakian (190). His studies showed that chloroplasts have a set of various enzymes present in different states, differing in the strength of their bonds with the plastid protein complex. The character of these bonds depends on the physiological state of the plant; it alters in its developmental cycle and can be disrupted upon the isolation of protoplasmatic structures and upon chloroplast destruction to grana. In chloroplasts enzymes are present that participate in oxido-reductive, hydrolytic, and various synthetic reactions (190). Frenkel's review (191) covers the data on chromatophore enzymic activity of photosynthetic bacteria.

The presence of various enzymes bound within the chloroplast structure is a prerequisite of their possible conjugation with photochemical pigment reactions; we are most interested in the systems able to catalyze reactions of the donor or acceptor molecules with electrons (hydrogen) mobilized in photochemical pigment reactions.

In the works of Hill (192), suggestions were made on the participation of a cytochrome system in the photosynthetic path of the electron. The works of Sissakian *et al.* (193) showed conjugation of cytochrome oxidase with photochemical chlorophyll reactions to be possible. Pyridine-nucleotides and flavins can be used as systems accepting electrons from chlorophyll. Participation of corresponding enzymes in this type of reactions is determined by dehydrogenase activity of plastids as revealed in the works of Sissakian *et al.* (194) and by the relation of this activity to the photochemical activity of chloroplasts studied with the use of the method of reversible inhibition (195). Apparently the "proteinic factor"—pyridine-nucleotide-reductase—mentioned below is an enzyme of this type. Boichenko & Zakharova (196) pointed out the hydrogenase activity of chloroplasts and probable role of the bound Fe and Mn in the reactions of CO_2 reduction. Particular attention is given at present to the conjugation of enzymes and photochemical reactions in phosphorylation.

Photochemical oxido-reductive reactions in homogenates of green leaves and chloroplasts.—Homogenates of leaves, as well as chloroplast suspensions are capable of the Hill reaction, as discussed in the extensive literature recently summarized, for example, in a review of Clendenning (197). In recent years attention has been drawn to simpler hydrogen transfer reactions in homogenates and chloroplasts. Sometimes the systems which have lost the

ability for the Hill reaction as a result of being kept at room temperature can undergo those simpler reactions (169). Apparently the sensitive enzymic systems participating in the liberation of the molecular oxygen are inactivated thereby.

Good & Hill (198) noticed photoreduction of FMN and benzylviologen by chloroplasts under strictly anaerobic conditions. A reversible photoreduction of safranin and an irreversible one of methyl red in homogenates of green leaves that contained sodium ascorbate was studied by our group (169). A reversible change of optical density in the chlorophyll absorption maximum in the presence of ascorbate was noticed in the same work; this change has to be ascribed to photoreduction.

Vernon & Hobbs (199) noted the photoreduction of FMN and indigocarmine in the presence of dichlorphenolindophenol, and ascorbate by homogenates of green sugar beet leaves. The authors associate this process with the Hill reaction, but in our opinion, in these reactions a chlorophyll-sensitized hydrogen transfer takes place with ascorbic acid playing the role of hydrogen donor.

Amidst compounds participating in these relatively simple reactions, it is cytochromes and pyridine nucleotides that attract a peculiar attention. Cytochrome transformations sensitized by chlorophyll were observed both in simple model systems (133), and in homogenates, chloroplast suspensions and living organisms. Extensive literature is devoted to this problem which was analyzed in recent reviews of Kamen (200), and Smith & Chance (201). Cytochromes can play the part of both the donor and acceptor of electrons in sensitized reactions in different systems. In relation to oxido-reductive properties of the medium, either photochemical reduction or oxidation of cytochromes is possible, both processes of which could be a step on the path of electron transfer in photosynthesis. When analyzing the mechanism of these reactions, suggestions were made on the transmission of the excitation energy from Mg to Fe-porphyrins; however, electron transfer is more likely in these cases (133).

Photoreduction of pyridine-nucleotides.—These reactions have drawn much attention, since reduced pyridine-nucleotides can participate in carbon dioxide assimilation.

In 1949 we have experimentally shown the possibility of photosensitized reduction of diphosphopyridine-nucleotide in solution, at the expense of light energy absorbed by chlorophyll (180, 181). In 1951 the papers of Vishniac & Ochoa (202), Tolmach (203), and Arnon (204) were published and showed the possibility of the reduction of pyridine-nucleotides in leaf homogenates or chloroplasts in the presence of enzymic preparations. San Pietro (205) and San Pietro & Lang (205a) with the use of spectrophotometric technique demonstrated pyridine-nucleotide reduction in homogenates and found a protein factor catalyzing the light reaction. The same conclusions were reached by Arnon *et al.* (206). Vernon *et al.* noticed photoreduction of pyridine-nucleotides in homogenates of photosynthetic bacteria

(207) and upon prolonged illumination of chloroplast suspension (199). Frenkel found the reduction of pyridine-nucleotides with the use of reduced FMN in the chromatophores of photosynthetic bacteria (208).

It was already mentioned that in the works of Sissakian *et al.* a correlation was revealed between the inhibition of pyridine-nucleotide dehydrogenases by nicotinamide and photochemical activity of chloroplasts (195).

In a number of the above mentioned papers the reduction of pyridine-nucleotides was judged by the increase of the extinction coeffcient at 340 mμ after illumination and by the drop of its value upon the addition of alcohol dehydrogenase and acetaldehyde as oxidation substrates. No measuring of absorption spectra of the reduced forms of pyridine-nucleotides could be done in this work, apparently, due to the strong light absorption by homogenates in this spectral region. Duysens & Amesz (209) observed pyridine-nucleotide reduction directly in living leaves with the application of differential spectroscopy and fluorescence measurement. Measuring fluorescence spectra of leaves at different temperatures, our group was able (22) to find maxima probably corresponding to bound and free forms of reduced pyridine-nucleotides.

The Tamyia group (210) did not find correlation between the reduced pyridine-nucleotides and the accumulation of the "reducing power" after the illumination of *Chlorella* cells (measured by $C^{14}O_2$ incorporation). An increase of TPN and a decrease of DPN were found upon illumination. The authors reach the important conclusion that in the experiments with preillumination the primary reducing agent is formed prior to the reduction of pyridine-nucleotides.

The elementary mechanism of the reduction of pyridine-nucleotides in chloroplasts seems to be connected to chlorophyll participation in the processes of electron transfer. However, the existence of this mechanism in biologically organized systems requires direct experiments.

*Photophosphorylation.**—It was often supposed that the primary photoproducts associated with pigment transformation led to the formation of substances participating in the dark biochemical reactions of photosynthesis. In papers of Warburg (213, 213a) ideas are developed on the formation of primary photoproducts that undergo back reactions connected with oxygen liberation and CO_2 assimilation; an analysis of these works is presented in the paper of Gaffron & Rosenberg (214). Experimental material now available suggests that the intermediates that store light energy after the photochemical stage are compounds well known to biochemists, such as reduced forms of pyridine-nucleotides, and high energy phosphates. It is, however, very likely that further investigations will lead to the discovery of new intermediate compounds [cf. works of Tamyia group (210)].

* Recent reviews of Arnon (211, 212) and a paper of Kandler in this issue were devoted to this problem. Therefore in the present review this question is considered very briefly.

Ideas concerning possible formation of high energy phosphates in photosynthesis were forwarded as early as in 1943 in the papers of Ruben (215) and Emerson *et al.* (216).

Wassink *et al.* (217) observed alterations in the amount of phosphate in suspensions of *Chromatium* and *Chlorella* with alternating dark and light. Kandler (218) found changes in phosphate amounts in *Chlorella* in the dark and upon illumination. Frenkel (219) noticed that illumination of photosynthetic bacteria under anaerobic conditions results in the storing of high energy phosphates. The works of Sissakian (190) and of others showed chloroplasts to be the focus not only of pigments but of various enzyme systems able to perform many biochemical syntheses as well.

Arnon, Allen & Whatley (220, 220a) discovered the ability of isolated chloroplasts to synthesize ATP in the light. The fact that washed chloroplasts have a very low respiration makes it easier to study formation of active compounds in question upon illumination. Detailed study of this phenomenon in Arnon's laboratory led to the following results: Upon chloroplast illumination, incorporation of inorganic phosphate (P) into ATP took place under conditions in which neither liberation nor uptake of oxygen was ever observed. Arnon called this reaction the "cyclic phosphorylation":

$$\text{light} + n\text{P} + n\text{ADP} \rightarrow n\text{ATP}$$

The rate of the process increased greatly if cofactors, such as ascorbic acid, FMN, derivatives of vitamin K (221), phenazine methosulfate (222), or some other oxido-reductive systems, capable of electron transfer were added. Hill & Walker (223) showed that phenazine methosulfate activity is due to its fast conversion into pyocyanine in light.

Presumably, cofactors become involved in the system of electron transfer between the photochemically active, reduced and oxidized compounds formed thereby. Further works of the Arnon group showed that the conditions of conjugation of the phosphorylation and the Hill reaction could be found according to the following summary equation:

$$2\text{FeCy}^{+3} + 2\text{H}_2\text{O} + \text{ADP} + \text{P} \rightarrow 2\text{FeCy}^{+2} + 1/2\text{O}_2 + \text{ATP}$$

and with the reaction of TPN reduction:

$$\text{TPN} + 2\text{H}_2\text{O} + \text{ADP} + \text{P} \rightarrow \text{TPNH}_2 + \text{ATP} + 1/2\text{O}_2 + \text{H}_2\text{O}$$

Here the extrinsically introduced electron acceptor reacts with the photochemically formed reductant, while oxygen is liberated in the "usual" photosynthetic way. Jagendorf analysed conditions of the involvement of electron carriers in reactions of different types (224). As a result of the second reaction given above, compounds are formed that are required for the cycle of CO_2 assimilation. This reaction occurs with small amounts of cofactors, such as FMN, vitamin K, ascorbic acid, or without them. With the addition of

cofactors, ATP accumulation sharply increased, due to cyclic phosphorylation, while oxygen liberation and TPN reduction were eliminated.

ATP formation when using the Hill oxidants as electron acceptors is readily understandable, as in this case the "energy overfall" is large; the E_o' of primary photoreduced compounds does not exceed the potential of the hydrogen electrode (−0.42v. at pH 7); their interaction with the system Fe^{+++}/Fe^{++} (+0.78v.) is accompanied with a considerable decrease of ΔF of the system.

The situation is different when TPN or DPN ($E_o' - 0.32$v.) are electron acceptors; the "energy overfall" is small here and ATP formation can hardly be perceived on the way of electrons from the primary reductant to TPN, according to Arnon's scheme. Perhaps ATP is still formed in this case at the expense of the oxidation of the photoreduced TPN. It should be noted in this connection that an energetic TPNH and DPNH photooxidation, sensitized by chlorophyll in solutions and suspensions of chloroplasts and grana, was recently observed in our laboratory (181).

The Arnon group (211, 212) was able to separate the light and dark stages of the reaction: formation of the "assimilatory power," (the TPNH and ATP) upon illumination of green "grana" in the absence of oxygen; and the assimilation of labelled CO_2 upon interaction of the illuminated suspension with the stroma of chloroplasts in the dark. In the presence of catalytic amounts of glucose phosphate, phosphate esters of sugars and other compounds were obtained that were identified by means of chromatography and radioautography. These results were proved by experiments in which artificially obtained ATP and TPN were added to homogenates of green leaves with labelled CO_2.

These works serve as a basis for the pattern of photosynthesis that is now generally accepted: formation of reduced pyridine-nucleotides and high energy phosphates in light, and dark reactions of CO_2 assimilation with the participation of these compounds. However, the conjugation mechanism of these reactions with the primary photochemical process occurring with chlorophyll is, thus far, not clear.

One may forward the following hypothesis based on the study of the photochemical properties of chlorophyll (225). As a result of the primary photochemical process the acceptance of an electron by the excited chlorophyll molecule from the donor molecule (in the end, from a water molecule) takes place. The primary reductant (photoreduced chlorophyll?) and the oxidized hydrogen donor are formed in the midst of the lipid phase or at the phase boundary. Biochemical electron carriers, FMN, and vitamin K, can participate in back reactions with these active compounds.

It should be noted that the compounds of these types (riboflavins, quinones) readily react with the photoreduced form of chlorophyll, being reduced with the regeneration of the "green" chlorophyll. Introduction of an "irreversible" electron acceptor (the Hill oxidant or pyridine-nucleotides) directs the process towards the conjugation of the Hill reaction with ATP

formation while various "reversible" redox-systems "short-circuit" the cycle leading only to ATP formation. Thus, in all variants, phosphorylation is conjugated with photochemical electron labilization in the chloroplast grana and depends on the nature of intermediate systems transferring the electron.

Study of Intermediates by Means of Tracers

To study subsequently formed intermediates, tracers are widely applied. The organism photosynthesizes in the medium containing labelled molecules of CO_2, H_2O or other compounds; varying the periods of the darkness and illumination the organism is fixed (killed), and the distribution of isotope in the products formed and in atomic groups of labelled molecules is studied. An important stage in this procedure is the method of fixation in the process of which a distortion of the true isotope distribution is possible as a result of the destruction of unstable labelled compounds upon heating under the action of solvents, etc. Thereby the isotope can pass into more stable compounds detected analytically which are not really formed in living plants and are artifacts. The "mild" fixation methods of plant material were given great attention in recent works with labelled carbon in which primary products of photosynthesis were investigated (226 to 228).

The path of carbon.—The long-lived radioactive isotope of carbon, C^{14}, in the form of labelled carbon dioxide is now widely applied to the study of the products of carbon dioxide assimilation by plants. Only a few works were carried out using the heavy isotope C^{13}; the short-lived radioactive isotope C^{11} is not used at all at present.

A vast amount of study was devoted to the intermediates of photosynthesis, using $C^{14}O_2$. Our survey is not aimed to review these works, the more so, as there are modern monographs on this subject (229, 230). We would like to make only some brief comments. The process begins with the preliminary unstable binding of carbon dioxide [see Doman (231); Metzner *et al.* (232)]. Widely known works of Calvin, and Benson *et al.* showed that carbon dioxide reduction by plants is a multi-step cyclic process. In this cycle a compound is regenerated that binds carbon dioxide. According to Calvin's works, this compound is ribulose diphosphate which is then converted to phosphoglyceric acid. These results received confirmation in Doman's experiments (228) with low temperature fixation and chromatography of the primary products of carbon dioxide binding. The intermediates of the cycle and the nature of enzyme systems that regulate separate stages of the reaction become ever more clarified.

When studying the paths of carbon in photosynthesis, Boichenko & Zakharova (226) found iron to be present in the composition of primary products of $C^{14}O_2$ reduction.

The works of Nichiporovich *et al.* (233 to 235) showed that the relative rapidity of the entry and distribution of labelled carbon, assimilated in the process of photosynthesis, in various products (carbohydrates, amino acids, organic acids, proteins) varies strongly with the type and species of plant,

its age, the character of root and nitrogen nutrition, and the intensity and spectral composition of light.

Thus, red light promotes increased carbohydrate formation, while blue promotes formation of amino acids and proteins. Having noticed that blue light activates cytochrome, flavin systems, and respiration in light, the Nichiporovich group supposed that one of the causes altering the ratio of carbon transformations was the alteration of the ratio of oxidative to reductive reactions of photosynthesis.

The problems of the relation between photosynthesis and respiration are analysed in the recent review of Kolesnikov (236).

Unlike photosynthesis in plants, no sugars are stored as the final products during bacterial photosynthesis. Fuller (237) showed that in *Chromatium,* fixed carbon is primary accumulated in aspartic acid. This paper analyses the paths of carbon in bacterial photosynthesis.

The working of carbon cycles requires a supply of active products formed as a result of photochemical reactions. According to the above mentioned investigations, these are the reduced pyridine-nucleotides and ATP. Having given up their hydrogen in the carbon cycle, TPN and DPN return to the system of photochemical reactions; in a similar manner, ADP returns to the cycle of photosynthetic phosophorylation.

The path of hydrogen.—The heavy isotope of hydrogen (deuterium) and the radioactive one (tritium) were used in the form of heavy water and T_2O. Due to great differences in atomic weight of these isotopes from hydrogen, the value of isotopic discrimination is great here.

Moses & Calvin (238) showed that during *Chlorella* photosynthesis in the presence of T_2O and $C^{14}O_2$, T appeared in the same photosynthetic products as C^{14}. Since T of high specific activity was used, action of radioactivity was evaluated by the character of T distribution in assimilation products in these experiments as compared to control ones. It was noticed that T entered in light with an intensity three times that in the experiments in the dark. Primary T accumulation was noticed in glycolic, phosphoglyceric, and glutamic acid. In the dark experiments, T is accumulated to a greater extent in amino acids. The authors stress the difficulties in the interpretation of the results obtained due to hydrogen exchange and isotopic effect.

In other work, the Calvin group (239) studied the influence of high concentrations of D_2O in water (up to 9 per cent) on *Chlorella* photosynthesis). With the diminution of photosynthetic activity in this medium, the amount of the alcohol-insoluble fraction (proteins, polysaccharides, nucleic acids) diminished, and $C^{14}O_2$ distribution resembled carbon dioxide assimilation in the dark.

In order to elucidate the possibilities of a photochemical exchange of hydrogen atoms in the chlorophyll molecules in the process of photosynthesis, Kutyurin (67, 240) carried out his work on *Elodea canadensis* with a D_2O concentration in the nutritive medium from 5 to 20 per cent. N^{15} was used

simultaneously to determine the intensity of chlorophyll biosynthesis under the conditions of prolonged experiments. Chlorophyll extracted from plants was carefully purified by means of chromatography and dried to constant weight. Dry chlorophyll samples were burned and the water thus obtained was analysed by means of a mass spectrometer. In short-term experiments (2.4 hr.) no D was found in chlorophyll, while in 68-hour experiments the content of D corresponded to the equilibrium exchange of 1.3 hydrogen atoms per chlorophyll molecule.

The absence of a rapid photochemical exchange of hydrogen atoms in chlorophyll proved the lack of reversible dehydration of its molecule as a stage in photosynthesis. The possibility of deuterium storage in chlorophyll at the expense of pigment exchange and biosynthesis during the experiment is discussed in the paper (240). Vishniac & Rose (241) studied tritium incorporation in chlorophyll upon illumination of *Chlorella* and *Scenedesmus* in water containing T_2O. Experiments showed chlorophyll to contain only a minor part of tritium when compared to the total exchange (one hydrogen atom per one chlorophyll molecule). Thus, these results are in general conformity with previous experiments of Norris, Ruben & Allen (242).

However, Vishniac found that upon pigment extraction from chloroplast fragments by acetone at minus 5°C., some nonextracted chlorophyll remains in the residue. In these preparations chlorophyll easily exchanges hydrogen for tritium after illumination of algae. If these preparations are treated with alkali, the whole of the radioactivity passes to water. Thus, in the minor part of chlorophyll, which is present in some different form, the hydrogen is readily exchangeable. It is thus far not clear whether the incorporation of tritium proceeds at the expense of oxido-reductive, or acid-base processes. The latter seems more likely, as in model experiments, (chlorophyll in a mixture of pyridine with ethers and T_2O) incorporation of a certain amount of tritium into chlorophyll was observed. In pyridine, a labilization of the hydrogen atom at C_{10} of the chlorophyll molecule seems to take place. Vishniac (243) pointed out that upon paper chromatography of pigments from acetone powder, most tritium is revealed not in the zone of chlorophyll-*a*, but in the zone of another, as yet undetermined, pigment. It might be a chlorophyll derivative containing free carboxyl groups (without phytol).

Thus, the known experimental material obtained with the use of hydrogen isotopes shows the impossibility of chlorophyll participation in photosynthesis by means of the primary reversible dehydrogenization.

The path of oxygen.—Water labelled with the heavy oxygen isotope O^{18} was used by Ulubekova (244) to reveal the differences in oxygen metabolism during photosynthesis and photoreduction. The algae, after illumination in H_2O^{18} (in some cases $CO_2{}^{18}$ was used), were killed with liquid nitrogen and dried in vacuum. Dry cells were burned and the oxygen of the water thus obtained was analysed by mass spectrometry. Intensity of O^{18} incorporation in the organic matter of cells was in proportion to light in-

tensity; at 2 to 3 thousand lux no difference was found in the level of oxygen enrichment in photosynthesis and photoreduction.

The employment of the activation technique in the experiments of Calvin *et al.* (245) with O^{18} is very promising. This method is based on the irradiation of the preparations containing O^{18} with protons of the energy of 4.5 Mev. Thereby a radioactive isotope of fluorine, F^{18}, is formed. An experiment was carried out on eliminating the radioactive background, as a result of which conditions were found for the exact determination of products containing O^{18} after the illumination of algae in H_2O^{18}. The method, by its sensitivity, exceeds that of mass spectrometry, allowing determination of down to 0.1γ of O^{18} in the product upon incubation of algae in 20 per cent H_2O^{18}.

The heavy oxygen was shown to be accumulated during photosynthesis first in sugar-phosphates: mono- and diphosphate and phosphoglyceric acid. As yet, however, no intermediates were found prior to molecular oxygen liberation.

The improvement of the mass-spectrometric technique allowed it to be used for the registration of natural deviations in isotopic composition of oxygen and for the gas exchange evaluation (CO_2 uptake, O_2 liberation). Vinogradov *et al.* (246) measured the precise isotopic composition of the oxygen liberated in photosynthesis of *Elodea canadensis* and diatoms.

It was stated that respiration affects the isotopic composition of oxygen in photosynthesis. This phenomenon, which is due to the prevailing uptake of the isotope O^{16} in respiration, was studied in general form by Lane & Dole (247). Vinogradov *et al.* suggest that this effect can account for the difference in the isotopic composition of oxygen in photosynthesis and that of water, which attained as much as 10 to 20 per cent towards the enrichment of photosynthetic oxygen by O^{18} isotope in their experiments.

To sum up, the use of the isotope C^{14} allowed the general pattern of the path of carbon in photosynthesis to be stated; the use of the isotopes of hydrogen and oxygen for the study of intermediates of water photooxidation has given, thus far, no decisive results. One of the main problems of photosynthesis, the mechanism of molecular oxygen liberation, remains unsolved. Presumably, the intermediates of water oxidation are exceedingly short-lived, and the stage of molecular oxygen formation proceeds at a very high velocity. There are but few data on the participation of manganese in the system of these reactions (248).

Conclusion

Extensive experimental material obtained in the study of photosynthesis during the last five years gave further support to the following general pattern of the process of photosynthesis.

As a result of light quantum absorption by the pigment system of the plant, there takes place the primary photophysical act—formation of chlorophyll excited states of different lifetimes—and the appearance of unpaired

electrons, probably at the expense of the formation of the triplet states, and of the reversible electron acceptance by the excited chlorophyll molecules.

In the primary photochemical process, the excitation energy is changed into potential chemical energy of primary photoproducts. It is most likely that the primary photoprocess consists in the uplifting (in the terms of energy) of electrons (hydrogen) from donor molecules to acceptor molecules. Thereby the excited chlorophyll molecule can play the part of an intermediate electron (hydrogen) carrier, undergoing reversible photoreduction and regeneration in the cycle. It is not clear as yet whether the water molecule is involved in the primary photochemical process or in subsequent dark stages. No intermediates of water photooxidation have been found up to the present time.

Chlorophyll can pass the electron (hydrogen) from the donor molecule to those of the acceptor up to the level of reduced pyridine-nucleotides, while back reactions of primary photoproducts can be associated with the formation of high energy phosphates. Chloroplast enzymes participate, probably, in these reactions.

Thus, as a result of photochemical reactions coupled with the dark stages, intermediates of longer lifetimes are accumulated that "store" the light energy—reduced pyridine-nucleotides and high energy phosphates consumed in the further biochemical reactions of carbon dioxide reduction.

CO_2 assimilation in photosynthesis is a multi-stage cyclic enzymic process in which the compounds primarily binding CO_2 undergo regeneration. This cycle is closely coupled with other paths of plant metabolism, such as respiration, nitrogen assimilation, etc.

The above is only a vague outline of the most probable and accepted working hypothesis; the ultimate elucidation of the mechanism of photosynthesis requires much more effort.

LITERATURE CITED

1. Wolken, J., *Ann. Rev. Plant Physiol.*, **10**, 71 (1959)
2. Wassink, E. C., Katz, E., and Dorrenstein, R., *Enzymologia*, **7**, 113 (1939)
3. French, C. S., *J. Gen. Physiol.*, **23**, 483 (1940)
4. Vermeulen, D., Wassink, E. C., and Reman, H., *Enzymologia*, **4**, 254 (1937)
5. Duysens, L. N. M., *Nature*, **168**, 548 (1951)
6. Krasnovsky, A. A., Voinovskaya, K. K., and Kossobutskaya, L. M., *Dokla. dy Akad. Nauk S.S.S.R.*, **85**, 389 (1952)
7. Krasnovsky, A. A., Kossobutskaya, L. M., and Voinovskaya, K. K., *Dokla. dy Akad. Nauk S.S.S.R.*, **92**, 1201 (1953)
8. Brill, C., *Biochim. et Biophys. Acta*, **29**, 458 (1958)
9. Duysens, L. N. M., Huiskamp, W. J., Vos, J. J., and van der Hart, J. M., *Biochim. et Biophys. Acta*, **19**, 188 (1956)

9a. Duysens, L. N. M., *Carnegie Inst. Wash. Year Book*, **52**, 157 (1952–53)

10. Goedheer, J. C., *Carnegie Inst. Wash. Year Book*, **57**, 295 (1957–1958)
11. Voinovskaya, K. K., and Krasnovsky, A. A., *Biokhimya*, **18**, 1626 (1953)

11a. Krasnovsky, **A.** A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.* **81,** 879 (1951)
12. Weigl, J. W., *J. Am. Chem. Soc.*, **75,** 999 (1952)
13. Holt, A. S., and Jacobs, E. E., *Am. J. Botany,* **41,** 718 (1954)
14. Jacobs, E. E., Vatter, A. E., and Holt, A. S., *Arch. Biochem. Biophys.,* **53,** 228 (1954)
15. Komen, J. G., *Biochim. et Biophys. Acta,* **22,** 9 (1956)
16. Krasnovsky, A. A., *Biofizika,* **4,** 3 (1959)
17. Katz, E., and Wassink, E. C., *Enzymologia,* **7,** 108 (1939)
18. Larsen, H., *Kgl. Norske, Videnskab. Selskabs, Forh. Skrifter 1, 1953* (Trondheim 1954)
19. Krasnovsky, A. A., and Pakshina, E. V., *Doklady Akad. Nauk S.S.S.R.,* **127,** 913 (1959)
19a. Shaposhnikov, V. N., Kondratyeva, E. N., and Fedorov, V. D., *Mikrobiologia,* **27,** 529, 1958
20. Smith, J. H. C., and Benitez, A., *Carnegie Inst. Wash. Year Book,* **52,** 149, 153 (1952–53)
21. Shibata, K., *J. Biochem. (Tokyo),* **44,** 147 (1957)
22. Litvin, F. F., and Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.,* **117,** 106 (1957); *Doklady Akad. Nauk S.S.S.R.,* **120,** 764 (1958); *Izvest. Akad. Nauk S.S.S.R., Ser. fizicheskaia,* **23,** 82 (1959)
23. Krasnovsky, A. A., and Kossobutskaya, L. M., *Doklady Akad. Nauk S.S.S.R.,* **85,** 177 (1952)
24. Kossobutskaya, L. M., and Krasnovsky, A. A., *Biokhimja,* **19,** 37 (1954)
25. Krasnovsky, A. A., and Bystrova, M. I., *Biokhimja,* **25,** 1 (1960)
26. Litvin, F. F., Krasnovsky, A. A., and Rikhireva, G. T., *Doklady Akad. Nauk S.S.S.R.,* **127,** 699 (1959)
27. Smith, J. H. C., and Benitez, A., *Carnegie Inst. Wash. Year Book,* **52,** 148 (1952–53)
28. Smith, J. H. C., and Kupke, D. W., *Nature,* **178,** 751 (1956)
29. Smith, J. H. C., and French, C. S., *Carnegie Inst. Wash. Year Book,* **57,** 190 190 (1957–58)
30. Latimer, P., and Smith, J. H. C., *Carnegie Inst. Wash. Year Book,* **57,** 293 (1957–58)
31. Godnev, T. N., Shlyck, A. A., and Liakhnovich, J. P., *Fiziol. Rastenii Akad. Nauk S.S.S.R.,* **4,** 393 (1957)
32. Wolf, J., and Price, L., *Plant Physiol.,* **30,** Suppl. 31 (1956); *Arch. Biochem. Biophys.,* **72,** 293 (1957)
33. Löeffler, J. E., *Carnegie Inst. Wash. Year Book,* **54,** 159 (1954–55)
34. Krasnovsky, A. A., and Kossobutskaya, L. M., *Doklady Akad. Nauk S.S.S.R.,* **91,** 343 (1953)
35. Krasnovsky, A. A., Nesterovskaya, E. A., and Goldenberg, A. B., *Biofizika,* **1,** 328 (1956)
36. French, C. S., and Elliott, R. F., *Carnegie Inst. Wash. Year Book,* **57,** 278 (1957–58)
36a. French, C. S., *Photobiology,* 52 (Proc. Ann. Biol. Colloq., 19th Meeting, Oregon State College, Corvallis, Ore., April 1958)
37. Krasnovsky, A. A., and Kossobutskaya, L. M., *Doklady Akad. Nauk S.S.S.R.,* **104,** 440 (1955)

38. Vorobieva, L. M., and Krasnovsky, A. A., *Biokhimja*, **21**, 126 (1956)
39. French, C. S., and Church, A. B., *Carnegie Inst. Wash. Year Book*, **54**, 162 (1954–55)
40. French, C. S., and Huang, H. S., *Carnegie Inst. Wash. Year Book*, **56**, 266 (1956–57)
41. French, C. S., and Young, V. K., *J. Gen. Physiol.*, **35**, 873 (1952)
42. Emerson, R., Chalmers, R., and Cederstrand, C., *Proc. Natl. Acad. Sci. U.S.*, **43**, 133 (1957)
42a. Emerson, R., Chalmers, R., Cederstrand, C., and Brody, M., *Science*, **123**, 673 (1956)
43. Yocum, C. S., and Blinks, L. R., *J. Gen. Physiol.*, **41**, 1113 (1958)
44. Brody, S. S., and Brody, M., *Arch. Biochem. Biophys.*, **82**, 161 (1959)
45. Kossobutskaya, L. M., and Krasnovsky, A. A., *Biokhimya*, **18**, 340 (1953)
46. Godnev, T. N., Terentieva, M. V., and Parmon, K. N., *Izvest. Akad. Nauk Belorus. S.S.R.*, **1**, 93 (1949)
47. Godnev, T. N., and Akulovich, N. A., *Referaty nauchno-issled. rabot Instituta Biologii Akad. Nauk Belorus. S.S.R. za 1955*, str. 49 (Izdat. Akad. Nauk Belorus. S.S.R., Minsk, 1956)
48. Ossipova, O. P., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **4**, 28 (1957)
49. Krasnovsky, A. A., Vorobieva, L. M., and Pakshina, E. V., *Fiziol Rastennii Akad. Nauk S.S.S.R.*, **4**, 124 (1957)
50. Brown, J. S., and French, C. S., *Plant Physiol.*, **34**, 305 (1959)
51. Maslova, T. G., and Sapozhnikov, D. I., *Problemy fotosinteza. Sbornik trudov II. Vsesoyuznoi Konferentsii po fotosintezu, Moskwa, 1957* (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
52. Godnev, T. N., and Akulovich, N. K., *Doklady Akad. Nauk S.S.S.R.*, **120**, 1307 (1958)
53. Jacobs, E. E., Holt, A. S., and Rabinowitch, E., *J. Chem. Phys.*, **22**, 142 (1954)
54. Trurnit, H. J., and Colmano, G., *Biochim. et Biophys. Acta*, **31**, 434 (1959)
55. Belavtseva, E. M., Vorobieva, L. M., and Krasnovsky, A. A., *Biofizika*, **4**, 521 (1959)
56. Rabinowitch, E., Jacobs, E. E., Holt, A. S., and Kromhout, R., *Z. Physik*, **133**, 261 (1952)
57. Jacobs, E. E., Holt, A. S., Kromhout, R., and Rabinowitch, E., *Arch. Biochem. Biophys.*, **72**, 495 (1957)
58. Jacobs, E. E., Vatter, A. E., and Holt, A. S., *J. Chem. Phys.*, **21**, 2246 (1953)
59. Donnay, G., *Arch. Biochem. Biophys.*, **80**, 80 (1959)
60. Zill, L. P., Colmano, G., and Trurnit, H. J., *Science*, **128**, 478 (1958)
61. Takashima, S., *Nature*, **169**, 182 (1952)
61a. Brody, S. S., *Science*, **128**, 838 (1958)
62. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **95**, 611 (1954)
63. Shlyck, A. A., Lyakhnovich, J. P., Kaler, V. L., and Lipskaya, G. A., *Doklady Akad. Nauk Belorus. S.S.R.*, **2**, 349 (1958)
64. Godnev, T. N., and Shlyck, A. A., *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva 1955*, **12**, 358 (1956)
65. Shlyck, A. A., *Metod mechenykh atomov v izuchenii biosinteza khlorofilla*, Minsk (1956); *Doklady Akad. Nauk Belorus. S.S.R.*, **1**, 121 (1957)*

66. Turchin, F. V., Guminskaya, M. A., and Plyshevskaya, E. G., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **2,** 3 (1955)
67. Kutyurin, V. M., *Doklady Akad. Nauk S.S.S.R.*, **106,** 355 (1956)
67a. Gubbenet, E. R., and Bazhanova, N. V., *Doklady Akad. Nauk S.S.S.R.*, **105,** 586 (1955)
68. Sironval, C., and Kandler, O., *Biochim. et Biophys. Acta,* **29,** 359 (1958)
69. Wolken, J. J., and Mellon, A. D., *Biochim. et Biophys. Acta,* **25,** 267 (1957)
70. Rabinowitch, E., *Photosynthesis,* VII, part 1 (Interscience Publ. Inc., New York, N.Y., 1951)
71. Franck, J., *Research in Photosynthesis,* 142 (Interscience Publ. Inc., New York, N.Y., 1957); *Proc. Natl. Acad. Sci. U.S.*, **44,** 941 (1958)
72. Gurinovich, T. P., Ermolenko, I. N., Sevchenko, A. N., and Solovyev, K. N., *Optika i Spektroskopia,* **3,** 237 (1957); *Problemy Fotosinteza,* str. 107 (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
72a. Stupp, R., and Kuhn, H., *Helv. Chim. Acta,* **35,** 2469 (1952)
73. Kravtsov, L. A., and Gruzinsky, V. V., *Doklady Akad. Nauk Belorus. S.S.R.,* **1,** 103 (1957)*
74. Dmitrievsky, O. D., Ermolaev, V. L., and Terenin, A. N., *Doklady Akad. Nauk S.S.S.R.,* **114,** 751 (1957)
75. Brody, S. S., and Rabinowitch E., *Science,* **125,** 555 (1957)
76. Tumerman, L. A., *Doklady Akad. Nauk S.S.S.R.,* **117,** 605 (1957)
77. Franck, J., and Livingston, R., *J. Chem. Phys.*, **9,** 184 (1941)
78. Terenin, A. N., *Acta Physicochimica, U.R.S.S.*, **18,** 210 (1943)
79. Lewis, G. N., and Kasha, M., *J. Am. Chem. Soc.*, **66,** 2100 (1944)
80. Tollin, G., Fujimori, E., and Calvin, M., *Nature,* **181,** 1266 (1958)
81. Becker, R. S., and Kasha, M., *J. Am. Chem. Soc.*, **77,** 3669 (1955)
82. Terenin, A. N., *Problemy Fotosinteza,* str. 9 (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
83. Krasnovsky, A. A., *Voprosi khimich. kinetiki, kataliza i reaktsion. sposobnosti,* str. 92 (Izd. Akad. Nauk, S.S.S.R., 1955)
84. Strehler, B. L., and Arnold, W., *J. Gen. Physiol.*, **34,** 809 (1951)
85. Strehler, B. L., and Lynch, V., *Arch. Biochem. Biophys.*, **70,** 527 (1957)
86. Arthur, W. E., and Strehler, B. L., *Arch. Biochem. Biophys.*, **70,** 507 (1957)
87. Litvin, F. F., Vladimirov, G. A., and Krasnovsky, A. A., *Uspekhi Fiz. Nauk,* **71,** No. 1 (1960)
88. Tollin, G., Fujimori, E., and Calvin, M., *Proc. Natl. Acad. Sci. U.S.*, **44,** 1035 (1958)
88a. Tollin, G., and Calvin, M., *Proc. Natl. Acad. Sci. U.S.*, **43,** 895 (1957)
89. Arnold, W. A., and Sherwood, H. K., *Proc. Natl. Acad. Sci. U.S.*, **43,** 105 (1957)
90. Calvin, M., and Sogo, P. B., *Science,* **125,** 499 (1957)
91. Sogo, P. B., Pon, N. G., and Calvin, M., *Proc. Natl. Acad. Sci. U.S.*, **43,** 387 (1957)
92. Arnold, W. A., and Sherwood, H. K., *J. Phys. Chem.*, **63,** 2 (1959)
93. Terenin, A. N., and Ermolaev, V. L., *Uspekhi Fiz. Nauk,* **58,** 37 (1956)
93a. Terenin, A. N., *Uspekhi Fiz. Nauk,* **43,** 347 (1951)
94. Rabinowitch, E., *J. Phys. Chem.*, **61,** 870 (1957)
95. Arnold, W., and Meek, E. S., *Arch. Biochem. Biophys.*, **60,** 82 (1956)

96. Konev, S. V., *Problemy Fotosinteza,* str. 94 (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
97. Terenin, A. N., and Ermolaev, V. L., *Doklady Akad. Nauk S.S.S.R.,* **85,** 547 (1952)
98. Terenin, A. N., and Putzeiko, E. K., *J. chim. phys.,* **55,** 681 (1958)
99. Putzeiko, E. K., *Doklady Akad. Nauk S.S.S.R.,* **124,** 796 (1959)
100. Nelson, R. C., *J. Chem. Phys.,* **27,** 864 (1957)
101. Calvin, M., "The photochemical apparatus; its structure and function," *Brookhaven Symposia in Biol., No. 11,* 160 (1959)
102. Arnold, W., and Maclay, H. K., *Brookhaven Symposia in Biol.,* No. 11, 1 (1959)
103. Vartanyan, A. T., and Karpovich, I. A., *Zhur. Fiz. Khim.,* **32,** 178 (1958)
104. Commoner, B., Heise, J. J., and Townsend, J., *Proc. Natl. Acad. Sci. U.S.,* **42,** 710 (1956)
105. Commoner, B., Heise, J. J., Lippicott, B. B., Norberg, R. E., Passonneau, J. V., and Townsend, J., *Science,* **126,** 57 (1957)
106. Sogo, P. B., Jost, M., and Calvin, M., *Rad. Research,* **1,** 511 (1959)
107. Rabinowitch, E., and Weiss, J., *Proc. Royal Soc. (London) A,* **162,** 251 (1937)
108. Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.,* **58,** 617, 835 (1947)
109. Krasnovsky, A. A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.,* **81,** 879 (1951)
110. Butsko, S. S., and Dain, B. J., *Zhur. Obshchei Khim.,* **28,** 2603 (1958); *Dopovidi Akad. Nauk Ukr. R.S.R.,* No. 11, 1221 (1958)
111. Dilung, I. I., *Ukrain. Khim. Zhur.,* **24,** 202 (1958)
112. Goedheer, J. C. (Doctoral thesis, Univ. of Utrecht, 1957); *Biochim. et Biophys. Acta,* **27,** 478 (1958)
113. Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.,* **60,** 421 (1948)
114. Krasnovsky, A. A., *Zhur. Fiz. Khim.,* **30,** 968 (1956); *J. chim. phys.,* **55,** 968 (1958); *Proc. Intern. Symp. Enzyme Chemistry,* Tokyo and Kyoto, Japan, 1957, p. 355 (1958); "The Origin of Life on the Earth," 351, *Repts. on the International Symposium,* August 1957, Moscow, U.S.S.R. (The Publishing House of the Acad. Sci. U.S.S.R., Moscow, U.S.S.R., 1957)
115. Krasnovsky, A. A., Brin, G. P., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.,* **69,** 393 (1949); **89,** 527 (1953)
116. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.,* **91,** 899 (1953)
117. Rabinowitch, E., *Photosynthesis,* **II,** part 2, 1519 (Interscience Publ. Inc., New York, N.Y., 1951)
118. Linschitz, H., and Weissman, S. I., *Arch. Biochem. Biophys.,* **67,** 491 (1957)
119. Lynch, V. H., and French, C. S., *Carnegie Inst. Wash. Year Book,* **54,** 166 (1954–55)
120. Rackow, B., and König, H., *Z. Elektrochem.,* **62,** 482 (1958)
121. Bannister, T. T., *Dissertation Abstr.,* **19,** No. 10, 2438 (1959)
122. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.,* **74,** 781 (1950); **96,** 1201 (1954)
123. Evstigneev, V. B., *Zhur. Fiz. Khim.,* **32,** 969 (1958)
124. Holt, A. S., *Research in Photosynthesis,* 55 (Interscience Publ. Inc., New York, N.Y., 1957)

125. Krasnovsky, A. A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.*, **64,** 663 (1949)
126. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **118,** 1146 (1958); **124,** 691 (1959)
126a. Evstigneev, V. B., and Savkina, I. G., *Biofizika,* **4,** 290 (1959)
127. Krasnovsky, A. A., and Pakshina, E. V., *Doklady Akad. Nauk S.S.S.R.*, **127,** 913 (1959)
128. Krasnovsky, A. A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.*, **96,** 1209 (1954)
129. Krasnovsky, A. A., and Pakshina, E. V., *Doklady Akad. Nauk S.S.S.R.*, **120,** 581 (1958)
130. Krasnovsky, A. A., and Umrikhina, A. V., *Doklady Akad. Nauk S.S.S.R.*, **122,** 1061 (1958)
131. Ashkinazi, M. S., and Dain, B. J., *Doklady Akad. Nauk S.S.S.R.*, **80,** 385 (1951); **102,** 767 (1955)
132. Dain, B. J., and Ashkinazi, M. S., *Problemy Fotosinteza,* str. 55 (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
132a. Vostrilova, N. V., and Dulova, V. I., *Doklady Akad. Nauk Uzbekskoi S.S.R.*, No. 5, 19 (1958)
133. Krasnovsky, A. A., and Voinovskaya, K. K., *Biofizika,* **1,** 120 (1956)
133a. Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.*, **103,** 283 (1955)
134. Krasnovsky, A. A., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **81,** 1105 (1951)
135. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **98,** 1017 (1954)
136. Krasnovsky, A. A., Drozdova, N. N., and Pakshina, E. V., *Biokhimya,* **25,** No. 2 (1960)
137. Krasnovsky, A. A., Evstigneev, V. B., Brin, G. P., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **82,** 947 (1952)
138. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **73,** 1239 (1950)
139. Rabinowitch, E., *J. Chem. Phys.*, **8,** 551, 560 (1940)
140. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **92,** 381 (1953)
141. Hendrich, W., *Roczniki chemii,* **32,** 107 (1958)
142. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **103,** 97 (1955)
143. Oster, G., *Research in Photosynthesis,* 50 (Interscience Publ. Inc., New York, N.Y., 1957)
144. Krasnovsky, A. A., and Umrikhina, A. V., *Doklady Akad. Nauk S.S.S.R.*, **104,** 882 (1955)
144a. Krasnovsky, A. A., and Umrikhina, A. V., *Biofizika,* **3,** 547 (1958)
145. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **96,** 1025 (1954)
146. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **115,** 530 (1957)
147. Porret, D., and Rabinowitch, E., *Nature,* **140,** 321 (1937)
148. Livingston, R., *J. Phys. Chem.*, **45,** 312 (1941); **52,** 622 (1948)

148a. Livingston, R., and Ryan, V., *J. Am. Chem. Soc.*, **75**, 2176 (1953)
148b. Livingston, R., *Research in Photosynthesis*, 3 (Interscience Publ. Inc., New York, N.Y., 1957)
149. Linschitz, H., and Rennert, J., *Nature*, **169**, 193 (1952)
150. Linschitz, H., and Abrahamson, E. W., *Research in Photosynthesis*, 31 (Interscience Publ. Inc., New York, N.Y., 1957)
151. Terenin, A. N., Karyakin, A. V., Lyubomudrov, E. B., Sushinsky, P. E., and Dmitrievsky, O. D., *Optika i spektroskopia*, **1**, 456 (1956)
152. Terenin, A. N., *Gen. Discussions of Faraday Soc.*, April 1959
153. Fujimori, E., and Livingston, R., *Nature*, **180**, 1036 (1957)
154. Krasnovsky, A. A., *Biofizika*, **4**, 3 (1959)
155. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **108**, 507 (1956)
156. Evstigneev, V. B., Gavrilova, V. A., and Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.*, **74**, 315 (1950)
157. Uri, N., *J. Am. Chem. Soc.*, **74**, 5808 (1952)
158. Rosenberg, I. L., *Ann. Rev. Plant Physiol.*, **8**, 115 (1957)
159. Commoner, B., Personal Communication at the 4th Intern. Congr. of Biochem. (Vienna, Austria, 1958)
159a. Bubnov, N. N., Tsepalov, V. F., Umrikhina, A. V., and Shlyapintokh, V. J., *Biofizika*, No. 2 (1960)
160. Latimer, P., and Rabinowitch, E., *Research in Photosynthesis*, 100 (Interscience Publ. Inc., New York, N.Y., 1957)
161. Latimer, P., *Plant Physiol.*, **34**, 193 (1959)
162. Duysens, L. N. M., *Science*, **120**, 353 (1954); *Research in Photosynthesis*, 59 (Interscience Publ. Inc., New York, N.Y., 1957)
163. Witt, H. T., *Z. Elektrochem.*, **59**, 981 (1955)
164. Bell, L. N., *Doklady Akad. Nauk S.S.S.R.*, **107**, 329 (1956)
165. Coleman, J. W., Holt, A. S., and Rabinowitch, E., *Science*, **123**, 795 (1956); *Research in Photosynthesis*, 68 (Interscience Publ. Inc., New York, N.Y., 1957)
166. Coleman, J. W., and Rabinowitch, E., *J. Phys. Chem.*, **63**, 30 (1959)
167. Kok, B., *Plant Physiol.*, **34**, 184 (1959); *Nature*, **179**, 583 (1957); *Acta Botan. Neerl.*, **6**, 316 (1957); *Biochim. et Biophys. Acta*, **22**, 399 (1956)
168. Witt, H. T., Moraw, R., and Müller, A., *Z. Naturforsch.*, **13b**, 822 (1958)
169. Vorobieva, L. M., and Krasnovsky, A. A., *Biokhimya*, **23**, 760 (1958)
170. Witt, H. T., *Research in Photosynthesis*, 75 (Interscience Publ. Inc., New York, N.Y., 1957)
170a. Witt, H. T., Moraw, R., and Müller, A., *Z. Phys. Chem.*, **12**, 393 (1957); **13**, 113 (1957)
171. Bell, L. N., and Chmora, S. N., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **6**, 91 (1959)
171a. Bell, L. N., *Doklady Akad. Nauk S.S.S.R.*, **113**, 695 (1957)
172. Duysens, L. N. M., *Research in Photosynthesis*, 164 (Interscience Publ. Inc., New York, N.Y., 1957)
173. Smith, L., and Ramírez, J., *Arch. Biochem. Biophys.*, **79**, 233 (1959)
174. Warburg, O., Krippahl, G., and Schröder, W., *Z. Naturforsch.*, **10b**, 631 (1955)

175. Emerson, R., *Ann. Rev. Plant Physiol.*, **9**, 1 (1958)
176. French, C. S., and Myers, J., *Carnegie Inst. Wash. Year Book*, **58**, 318 (1958–59)
177. Voskresenskaya, N. P., and Zak, E. G., *Doklady Akad. Nauk S.S.S.R.*, **114**, 375 (1957)
178. Krasnovsky, A. A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.*, **112**, 911 (1957)
179. Krasnovsky, A. A., *Doklady Akad. Nauk S.S.S.R.*, **61**, 91 (1948)
180. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **67**, 325 (1949)
181. Brin, G. P., and Krasnovsky, A. A., *Biokhimya*, **24**, No. 6 (1959)
182. Krasnovsky, A. A., and Voinovskaya, K. K., *Doklady Akad. Nauk S.S.S.R.*, **87**, 109 (1952)
183. Livingston, R., and Pariser, R., *J. Am. Chem. Soc.*, **70**, 1510 (1948)
184. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **58**, 1087 (1947)
184a. Brin, G. P., and Krasnovsky, A. A., *Biokhimya*, **22**, 776 (1957)
185. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **127**, 198 (1959)
186. Weiss, T., *Trans. Faraday Soc.*, **32**, 1331 (1936)
187. Krasnovsky, A. A., and Brin, G. P., *Doklady Akad. Nauk S.S.S.R.*, **53**, 447 (1946)
188. Evstigneev, V. B., and Terenin, A. N., *Doklady Akad. Nauk S.S.S.R.*, **81**, 223 (1951)
189. Evstigneev, V. B., and Gavrilova, V. A., *Doklady Akad. Nauk S.S.S.R.*, **126**, 410 (1959)
190. Sissakian, N. M., *Advances in Enzymol.*, **20**, 201 (1958); *Izvest. Akad. Nauk S.S.S.R., Ser. Biol.*, No. 5, 32 (1956)
191. Frenkel, A. W., *Ann. Rev. Plant Physiol.*, **10**, 53 (1959)
192. Hill, R., *Advances in Enzymol.*, **12**, 1 (1951)
193. Sissakian, N. M., Krasnovsky, A. A., Mikhailova, E. S., and Brin, G. P., *Biokhimya*, **24**, 3 (1959)
194. Sissakian, N. M., and Chamova, K. G., *Doklady Akad. Nauk S.S.S.R.*, **67**, 337 (1949)
195. Sissakian, N. M., Krasnovsky, A. A., Mikhailova, E. S., and Brin, G. P., *Biokhimya*, **18**, 725 (1953)
196. Boichenko, E. A., and Zakharova, N. N., *Fiziol. Rastenii, Akad. Nauk S.S.S.R.*, **6**, 88 (1959)
197. Clendenning, K. A., *Ann. Rev. Plant Physiol.*, **8**, 137 (1957)
198. Good, N., and Hill, R., *Arch. Biochem. Biophys.*, **57**, 355 (1955)
199. Vernon, L. P., and Hobbs, M. O., *Arch. Biochem. Biophys.*, **72**, 25 (1957)
200. Kamen, M., *Research in Photosynthesis*, 149 (Interscience Publ. Inc., New York, N.Y., 1957)
201. Smith, L., and Chance, B., *Ann. Rev. Plant Physiol.*, **9**, 449 (1958)
202. Vishniak, W., and Ochoa, S., *Nature*, **167**, 768 (1951); *J. Biol. Chem.*, **195**, 75 (1952)
203. Tolmach, L., *Arch. Biochem. Biophys.*, **33**, 120 (1951); *Nature*, **167**, 946 (1951)

204. Arnon, D. I., *Nature,* **167,** 1008 (1951)
205. San Pietro, A., *Science,* **124,** 118 (1956)
205a. San Pietro, A., and Lang, N. M., *J. Biol. Chem.,* **231,** 211 (1958)
206. Arnon, D. I., Whatley, F. R., and Allen, M. B., *Nature,* **180,** 182 (1957); *Science,* **177,** 1026 (1958)
207. Vernon, L. P., and Ihnen, E. D., *Biochim. et Biophys. Acta,* **24,** 115 (1957)
208. Frenkel, A. W., *J. Am. Chem. Soc.,* **80,** 3479 (1958)
209. Duysens, L. N. M., and Amesz, J., *Biochim. et Biophys. Acta,* **24,** 19 (1957)
210. Tamiko-oh-Hama, M. S., and Tamyia, H., *Proc. Intern. Botan. Congr. 9th Congr., Montreal, Canada, 1959,* 886 (University of Toronto Press, Canada 1959); *Biochim. et Biophys. Acta,* **34,** 202 (1959)
211. Arnon, D. I., Whatley, F. R., and Allen, M. B., *Science,* **127,** 3305 (1958)
212. Arnon, D. I., *Brookhaven Symposia in Biol.,* No. 11, 181 (1959)
213. Warburg, O., *Naturwissenschaften,* **42,** 449 (1955)
213a. Warburg, O., and Krippahl, G., *Z. Naturforsch.,* **10b,** 301 (1955)
214. Gaffron, H., and Rosenberg, J., *Naturwissenschaften,* **42,** 354 (1955)
215. Ruben, S., *J. Am. Chem. Soc.,* **65,** 279 (1943)
216. Emerson, R. L., Stanffer, J. F., and Umbreit, W. W., *Am. J. Botany,* **31,** 107 (1944)
217. Wassink, E. C., Wintermans, I. F., and Tjia, I. E., *Proc. Acad. Sci. Amsterdam,* **52,** 412 (1949); **54,** 41 (1951)
218. Kandler, O., *Z. Naturforsch.,* **5b,** 423 (1950)
219. Frenkel, A. W., *J. Am. Chem. Soc.,* **76,** 6324 (1954); *J. Biol. Chem.,* **222,** 823 (1956)
220. Arnon, D. I., Whatley, F. R., and Allen, M. B., *J. Am. Chem. Soc.* **76,** 6324 (1954)
220a. Arnon, D. I., Allen, M. B., and Whatley, F. R., *Nature,* **174,** 394 (1954)
221. Wessels, J. S. C., *Biochim. et Biophys. Acta,* **25,** 97 (1957); **29,** 113 (1958)
222. Jagendorf, A. T., and Avron, M., *J. Biol. Chem.,* **231,** 227 (1958)
223. Hill, R., and Walker, D. A., *Plant Physiol.,* **34,** 240 (1959)
224. Jagendorf, A. T., *Brookhaven Symposia in Biol., No. 11,* 236 (1959)
225. Krasnovsky, A. A., *Problemy Fotosinteza* (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
226. Boichenko, E. A., and Zakharova, N. I., *Biokhimya,* **21,** 374, 623 (1956)
227. Metzner, H., Simon, H., Metzner, B., and Calvin, M., *Proc. Natl. Acad. Sci. U.S.,* **43,** 892 (1957)
228. Doman, N. G., *Proc. Intern. Botan. Congr., 9th Congr. Montreal, Canada, 1958,* 94 (University of Toronto Press, Canada, 1959)
229. Bassham, J. A., and Calvin, M., *The Path of Carbon in Photosynthesis* (Prentice-Hall, Inc., Englewood Cliffs, N.Y., 1957)
230. Zalensky, O. V., Semikhatova, O. A., and Voznesensky, V. L., *Metodi primenenia radioaktivnogo ugleroda C^{14} dlia izuchenia fotosinteza* (Izd. Akad. Nauk S.S.S.R., Moskwa, Leningrad, 1955)
231. Doman, N. G., *Doklady Akad. Nauk S.S.S.R.,* **85,** 607 (1952); *Problemy Fotosinteza,* str. 394 (Izdat. Akad. Nauk S.S.S.R., Moskwa, 1959)
232. Metzner, H., Simon, H., and Metzner, B., *Z. Naturforsch.,* **13b,** 366 (1958)
233. Nichiporovich, A. A., *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955,* **12,** 340 (1956)

234. Nichiporovich, A. A., Andreeva, T. F., Voskresenskaya, N. P., Nezgovorova, L. A., and Novitzky, U. I., *Radioisotopes in Scientific Research*, **4**, 411; *Proc. UNESCO Intern. Conf., Paris, 1957, 1st Conf.* (Pergamon Press, London, England, New York, N.Y., and Paris, France, 690 pp., 1958)
235. Nichiporovich, A. A., and Voskresenskaya, N. P., *Proc. Intern. Botan. Congr. 9th Congr. Montreal, Canada, 1959*, 281 (University of Toronto Press, Canada, 1959)
236. Kolesnikov, P. A., *Uspekhi Sovremennoĭ Biol.*, **47**, 362 (1959)*
237. Fuller, R. C., *Proc. Intern. Botan. Congr., 9th Congr., Montreal, Canada, 1959*, 125 (University of Toronto Press, Canada, 1959)
238. Moses, V., and Calvin, M., *Biochem. J.*, **71**, 18 (1959)
239. Moses, V., Holm-Hansen, O., and Calvin, M., *Biochim. et Biophys. Acta*, **28**, 62 (1958)
240. Kutyurin, V. M., *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, **7**, No. 2 (1960)
241. Vishniac, W., and Rose, J. A., *Nature*, **182**, 1089 (1958)
242. Norris, T. H., Ruben, S., and Allen, M. B., *J. Am. Chem. Soc.*, **64**, 3037 (1942)
243. Vishniac, W., *Proc. Intern. Botan Congr., 9th Congr., Montreal, Canada, 1959*, 417 (University of Toronto Press, Canada, 1959)
244. Ulubekova, M. V., *Doklady Akad. Nauk S.S.S.R.*, **112**, 772 (1957); *Problemy Fotosinteza*, str. 14 (Izd. Akad. Nauk S.S.S.R., Moskwa, 1959)
245. Fogelstrom-Fineman, J., Holm-Hansen, O., Folbert, M. B., and Calvin, M., *Intern. J. Appl. Radiation and Isotopes*, **2**, 280 (1957)
246. Vinogradov, A. P., Kutyurin, V. M., Ulubekova, M. V., and Zadorozhny, I. K., *Doklady Akad. Nauk S.S.S.R.*, **125**, 1151 (1959)
247. Lane, G. A., and Dole, M., *Science*, **123**, 574 (1956)
248. Kessler, E., *Research in Photosynthesis*, 243 (Interscience Publ. Inc., New York, N.Y., 1957)

* English translation will be announced in Technical Translations, issued by the Office of Technical Services, U.S. Dept. of Commerce, and will be made available by the Photoduplication Service, Library of Congress and by the SLA Translation Center at the John Crear Library, Chicago, Illinois.

GIBBERELLINS AS NATIVE PLANT GROWTH REGULATORS[1,2]

BERNARD O. PHINNEY
Department of Botany, University of California, Los Angeles

CHARLES A. WEST
Department of Chemistry, University of California, Los Angeles

INTRODUCTION

The accumulation since 1955 of an exceedingly large number of publications on gibberellins has been accompanied by several reviews on the subject. The *Source Book of Gibberellins, 1828–1957,* published in 1958 by Stodola (1), is a valuable reference that includes a collection of more than 600 abstracts of papers pertaining to the general subject. Detailed and critical reviews on gibberellins have also appeared in 1957 and 1959, by Stowe & Yamaki (2, 3) and by Brian (4). The responses of economic plants to gibberellins have been covered recently by Wittwer & Bukovac (5).

Because of these recent reviews, we shall make no attempt to consider the subject of gibberellins in any comprehensive manner. Instead, certain aspects have been selected on the basis of their absence or lack of emphasis in previous reviews. The origin and present status of the gibberellin terminology will be considered; then suggestions will be made for a nomenclature that may help to minimize confusion in the future. The current information on structural studies of the gibberellins will be covered, as will evidence for the biosynthetic origin of gibberellin. A_3. The subjects of bioassays and of gibberellins as native plant growth regulators will be considered in some detail and the information on gibberellin-like substances will be used as a basis for discussing certain kinds of gibberellin-induced growth responses.

TERMINOLOGY

The historical development of information on native growth regulators is, in general, associated with the assignment of terms to chemically unknown substances that produced specific growth responses when added to an organism in minute amounts. As additional information on their biological and chemical properties is accumulated, the original use of the terms often becomes so modified and inclusive as to make the nomenclature quite unwork-

[1] The survey of the literature pertaining to this review was concluded in December, 1959.

[2] The following abbreviations will be used: GA-1 (gibberellin A_1), GA-2 (gibberellin A_2), GA-3 (gibberellic acid, gibberellin A_3), GA-4 (gibberellin A_4), GA-5 (gibberellin A_5); BF-I (bean factor I), BF-II (bean factor II), PF-I (pea factor I), PF-II (pea factor II), IAA (indolylacetic acid), PAA (phenylacetic acid).

able. The term *auxin* is a case in point; depending on the choice of definitions available in the literature, the term can include most plant growth regulators, even the gibberellins. Although the *Avena* curvature test (6) was originally the quantitative bioassay used to detect the presence of auxins, there are now numerous assays for this purpose (7). Even the chemically known auxin, IAA, was originally classified apart from a true auxin and named heteroauxin on the basis of the source from which it was originally isolated (8). Physiologically defined terms for growth regulators invariably become of limited practical value as additional information appears on their biological and chemical properties.

Gibberellins are known to produce many different types of growth responses (2, 3, 4); also the spectrum of responses induced by one gibberellin may differ both qualitatively and quantitatively from that of others (9). Finally, the growth responses currently thought to be induced only by the chemically known gibberellins, may ultimately be found to be associated with other substances quite unrelated in structure to the gibberellins. Thus, the choice of a bioassay for defining any unknown substance as a gibberellin becomes a difficult task. Since the chemical structure of at least five gibberellins is known with considerable certainty (10), there is at present a unique opportunity to use a terminology based on chemical and biological properties, and thus minimize the chance of the terms becoming obsolete in the future. While such a nomenclature was used to present evidence for substances from flowering plants having biological properties indistinguishable from the fungal gibberellins (11, 12, 13), specific suggestions for this kind of nomenclature have never been made in the literature. Indeed, it has been recommended that the term *gibberellin* be defined solely on the basis of biological properties (2); the suggestion has also been made for the discontinuance of the term *gibberellin-like* (14).

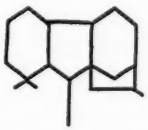
The origin of the term *gibberellin* is historically associated with the discovery that specific substances produced by the fungus *Fusarium moniliforme* Sheldon. [*Gibberella fujikuroi* (Saw.) Wr.] were responsible for the seedling elongation of *Oryza sativa* L. (rice) infected by this fungus. In 1912, Sawada (15) spoke of the "stimulation" from the fungus as causing rice seedlings to grow taller. In 1926, Kurosawa (16) called the active principle a "toxin." In later studies the expression "substance" or "growth promoting substance" was used to describe the biologically active material (17 to 23). The name *gibberellin* was given to this growth-promoting substance by Yabuta in 1935 (24); the material had been isolated as a fairly pure, but noncrystalline, solid from "bakanae" producing strains of the fungus. Thus, the term was originally assigned to a chemically unknown substance isolated from the fungus *Fusarium moniliforme,* which had the biological properties of producing "bakanae" symptoms, i.e., shoot elongation, in a rice seedling bioassay. The terms *gibberellin A* and *gibberellin B* were assigned in 1938 by Yabuta & Sumiki (25) to crystalline material obtained from their original gibberellin preparations. The terms were exchanged in later publica-

tions. Gibberellin B was probably a mixture of the biologically inactive allogibberic acid and biologically active gibberellins (*26, 27*); gibberellin A was probably a mixture of three biologically active gibberellins (*28, 29*). The first chemically pure gibberellin was named gibberellic acid in 1954 by Curtis & Cross (30) and gibberellin-X by Stodola in 1955 (31). At the present time five gibberellins have been isolated in chemically pure form and the following names have been assigned to these (10, *29, 32,* 33, 34): gibberellin A_1; gibberellin A_2; gibberellin A_3 (gibberellic acid, gibberellin-X); gibberellin A_4; and gibberellin A_5 (bean factor II). Thus, while the term gibberellin was originally defined by biological properties only, the five gibberellins isolated in pure form can be associated with similarities in chemical structure as well as similarities in biological properties.

The term *gibberellin-like* was originally assigned to substances obtained from a number of flowering plants that had the ability to produce normalcy of growth for certain single gene mutants of *Zea mays* L. (11, 12, 35). These mutants were known to exhibit a normal type growth response to the fungal gibberellins and no growth response to numerous other kinds of plant growth regulators. The term *gibberellic acid-like* was used by Radley (13, 36) for substances from flowering plants that had biological properties similar to gibberellin A_3 (gibberellic acid).

The following nomenclature is recommended and will be used in this review:

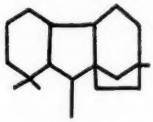
(*a*). *Gibberellin.* This term will be restricted to those substances that possess the same carbon skeleton as gibberellin A_3, or one very closely related to it, and are biologically active in stimulating cell division or cell elongation, or both, in plants (biological activity may be measured by changes in the dimension of organs or parts of organs). This would restrict the use of the term gibberellin to compounds of the type currently named as gibberellins. In line with the current designations of compounds of this group, it is suggested that a letter be adopted to indicate a particular carbon skeleton with a subscript number to indicate the specific compound with that carbon skeleton. Thus, the gibberellin A series would include the gibberellins GA-1, GA-*2*, GA-3, GA-4, and GA-5, all of which have the same carbon skeleton, as follows:



Gibberellin A series

The gibberellin C series would include the compound currently called gibberellin C, which is derived from gibberellin A_1 by an acid-catalyzed rearrangement. This might better be designated gibberellin C_1 according to the above

proposal. The acid-catalyzed rearrangement product derived from gibberellin A_5, which has a similar carbon skeleton, might then be called gibberellin C_5.



Gibberellin C series

According to this system, the biologically inactive gibberellin B of the Japanese (25) might better be referred to by the British name, allogibberic acid (26, 27).

(*b*). Gibberellin-like. This term will refer to substances having the biological property of inducing shoot elongation for certain cultivars, varieties, or mutants whose growth responses can be shown to be specific to the gibberellins. It is recommended that the type of plant chosen for bioassay possess a habit of growth known to be controlled by a single mutant gene. In theory, single gene dwarf mutants would be expected to be the most specific in their response to growth regulators (2, 37). Examples would be the dwarf mutants d_1, d_2, d_3, d_5, and an_1 of *Zea mays* (12, 38), and the dwarf mutant *le* of *Pisum sativum* L. (39, 40); the dwarf "Kidachi" cultivar of *Pharbitis nil* Chois., apparently controlled by a single mutant gene (41), and the gene-controlled rosetted variety of *Hyoscyamus niger* L., which is dependent on a cold requirement for elongation (bolting) and flowering (42, 43). Most dwarf cultivars and varieties are of unknown genotype. All citations for evidence of gibberellin-like substances should indicate the organism used for bioassay.

Chemistry of the Gibberellins

Gibberellins from the fungus Fusarium moniliforme.—The general properties of the four gibberellins, which were recovered from culture filtrates of *Fusarium moniliforme,* are given in Table I. Structural studies on these chemically pure gibberellins have progressed rapidly, chiefly through the recent efforts of one group of chemists located at the Akers and Butterwick Research Laboratories of Imperial Chemical Industries, Ltd. in England (44 to 51), and a second group in the Department of Agricultural Chemistry at the University of Tokyo (52 to 60). Gibberellin A_1 was shown to be dihydrogibberellic acid by the isolation of a methyl ester identical with the methyl ester of GA-1 from the reaction mixture resulting on partial hydrogenation of methyl gibberellate (55). Since gibberellin A_1 still possesses an exocyclic methylene group as indicated from ozonization studies (44, 53), the two hydrogens must have added to saturate the A ring. The English group has advanced structures I and II for GA-3 and GA-1, respectively (50), while the Japanese group has proposed structures III and IV for these

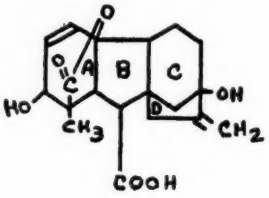
TABLE I

GIBBERELLINS FROM CULTURE FILTRATES OF *GIBBERELLA FUJIKUROI*

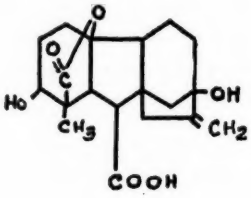
Name	Other names	*Molecular formula	*Molecular weight	M.P. C.	[α]D
Gibberellin A_3	Gibberellin "X" Gibberellic acid	$C_{19}H_{22}O_6$	346	232–235°	+86° †(0.51 in methanol, 20°C.) +92° †(1.74 in 95% ethanol, 25°C.)
Gibberellin A_1	Gibberellin "A" α-Dihydrogibberellic acid	$C_{19}H_{24}O_6$	348	255–268° (dec.)	+36° †(1.74 in 95% ethanol, 25°C.) +38° †(0.39 in ethanol, 20°C.)
Gibberellin A_2		$C_{19}H_{26}O_6$	350	—	—
Gibberellin A_4		$C_{19}H_{24}O_5$	332	222° (dec.)	−20.8°

* Indicated from elementary analysis and molecular weight determinations.
† Concentration in solvent and temperature.

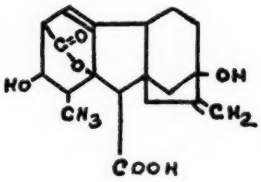
compounds (58). It will be noted that the proposals of the two groups differ only in the positions of attachment of the lactone ring in ring A. The Japanese have also advanced structure V for GA-2 and structure VI for GA-4 (59, 60). Thus, GA-2 differs from GA-1 in the position of the tertiary hydroxyl as well as in the lack of any ethylenic double bond, and is not simply dihydrogibberellin A_1. Gibberellin A_4 is identical with GA-1 except for the absence



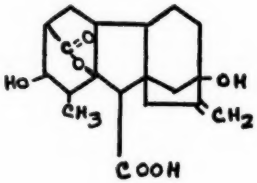
I



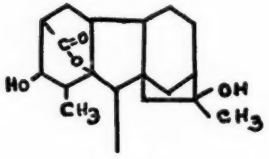
II



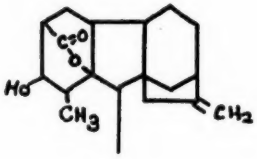
III



IV



V



VI

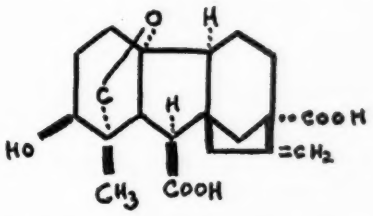
of the tertiary alcohol group. All these gibberellins have the same carbon skeleton.

The formulation of the ring skeleton and the positions of substitution of the methyl, carboxyl, secondary, and tertiary hydroxyls, and exocyclic methylene groups of gibberellic acid came from structural studies on the acid-catalyzed degradation products allogibberic acid (47) and gibberic acid (45), and fluorene derivatives formed on selenium catalyzed dehydrogenations (49, 56). A number of key degradation products were compared with synthetic materials (46, 48). The exact location of the lactone and the ethylenic double bond in the A ring has proved more difficult. The arguments of the English group for their proposed arrangement (49, 50) can be summarized

in part as follows: (*a*) the secondary alcohol is allylic to the double bond as determined from its oxidation to an α, β-unsaturated ketone with manganese dioxide in chloroform; (*b*) the lactone ring oxygen is allylic to the double bond as judged from the ease of hydrogenolysis of the lactone; (*c*) the nuclear magnetic resonance spectrum is consistent with the presence of methyl bound to carbon bearing no hydrogens and also other features in the proposed structure; (*d*) the infrared spectrum suggests an γ-lactone; and (*e*) the carbon skeleton is one which can be visualized as biosynthetically derived from isoprene units in a fashion analogous to diterpenes (see biosynthesis below).

The Japanese have published several experimental findings that seem inconsistent with the arrangement of the A ring of structures I and II. For example, they report that a dehydration product of gibberellin A_1 methyl ester can be ozonized to yield formaldehyde (56). They also obtain 1,3-dimethylfluorene (reported to be identical with synthetic material) on dehydrogenation of a lithium aluminum hydride reduction product of a gibberellin A_1 degradation product (57). Ozonization of a gibberellin A_3 degradation product in which the A ring double bond is intact yields what is claimed to be a monobasic acid which is readily decarboxylated in acid solution (58). Therefore, they formulate the A ring as shown in structures III and IV. Although these structures more readily explain the above findings, they are quite inconsistent with a number of the British observations. Furthermore, Takahashi *et al.* are forced to rationalize some of their own data in less likely ways in order to propose these structures. [See, for example, the formation of a cyclic eight-membered acid anhydride (57) and the interpretation of the 1760 cm.$^{-1}$ infrared absorption maximum as due to a strained γ-lactone (58).]

A series of experimental facts and arguments for the stereochemical arrangement of gibberellin A_3 (61) and allogibberic and gibberic acids (62) have been outlined by Stork & Newman. Cross *et al.* have also discussed their consideration of the stereochemistry of gibberellin A_3 (51). Both groups agree that their data support the following spatial arrangement:

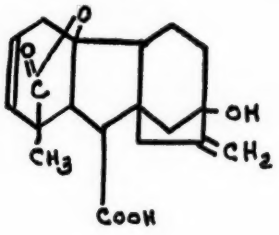


The dotted lines indicate groups projecting down (α) from the general plane of the ring system and the heavy lines indicate groups projecting up (β). The ring system is not actually planar, of course, and the steric relationships can

best be seen by the construction of models with these orientations of substituents.

Gibberellins from flowering plants.—Several groups have followed earlier reports of the occurrence of gibberellin-like substances in flowering plants with the isolation and identification of gibberellins from these sources. MacMillan & Suter have isolated gibberellin A_1 in small quantities from immature seed of *Phaseolus multiflorus* L. (63). West & Phinney extracted a substance, bean factor I, from immature seed of *Phaseolus vulgaris* L. (64) which subsequently was shown to be identical with gibberellin A_1 (34). Kawarada & Sumiki have also isolated gibberellin A_1 from "water sprouts" of *Citrus unshuii* (65). The yields of gibberellins in these cases were from 0.2 to 1.0 mg. per. kg. fresh weight of plant material. The identification in each case rested primarily on the identity of the infrared spectra of the free acid and the methyl ester of the isolated material with those of authentic gibberellin A_1.

West & Phinney obtained a second substance, bean factor II, from extracts of immature seed of *Phaseolus vulgaris,* which had gibberellin-like biological properties but differed in chemical and physical properties from the reported fungal gibberellins (64). MacMillan, Seaton & Suter (10) also obtained a second gibberellin, which they named gibberellin A_5, from their extracts of *Phaseolus multiflorus* seed. A comparison of infrared spectra of the gibberellin A_5 and bean factor II, and their methyl esters, has shown that these substances are identical. The name gibberellin A_5 has been accepted by both groups. MacMillan *et al.* propose structure VII for gibberellin A_5.



VII

Gibberellin A_5, therefore, corresponds to dihydrogibberellin A_1. A key reaction in this structural assignment was the dehydration of the acid-catalyzed degradation product of GA-1 (gibberellin C) to a product identical with the acid-catalyzed degradation product of GA-5. The findings of West are in agreement with this structural assignment (34).

Gibberellin A_3, the gibberellin produced in largest amounts in most fermentations, has not been isolated from flowering plants.[3] It might be expected,

[3] Baumgartner and his collaborators have obtained evidence by an isotope dilution technique for the presence of gibberellin A_3 in barley. These results have not as yet been published.

however, that gibberellin A_3 would be more difficult to isolate since it is more labile, particularly in acid solution. There is the suggestion from chromatographic evidence that still other gibberellins are present in plants (12).

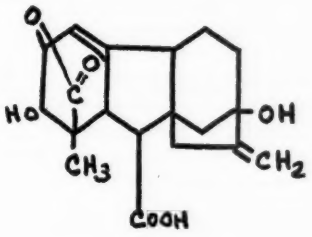
It is clear from evidence now at hand that gibberellins are present as natural products in low concentrations in flowering plants. This fact, coupled with the known physiological activities of gibberellins in flowering plants, strengthens greatly the hypothesis that they function in flowering plants as natural growth regulating substances.

Substances derived from the gibberellins.—In the course of structural studies, a number of derivatives of the gibberellins have been prepared and characterized.

Gibberellin A_3 is particularly sensitive to degradation in acid solution. Under mildly acidic conditions gibberellenic acid is formed by elimination of the lactone oxygen from the nucleus (Fig. 1) (66). Heating in acid solution leads to the complete aromatization of the A ring with the elimination of CO_2 and water to form allogibberic acid (gibberellin B), which in turn can be converted to gibberic acid in boiling mineral acid by a rearrangement in the C-D rings (26). The latter rearrangement has been pictured as a Wagner-Meerwein type by Mulholland *et al.* (47), or a hydration followed by a pinacol-pinacolone type by Birch *et al.* (67). None of these compounds retains biological activity (26, 27, 66).

Gibberellin A_1 undergoes a similar rearrangement in the C-D rings in acid solution to give gibberellin C (52, 53), but the saturated A ring is stable under these conditions. Gibberellin C retains some biological activity. Gibberellin A_2 and gibberellin A_4 are relatively stable to acid treatment.

Mild treatment of GA-1 and GA-3 in aqueous alkali not only can hydrolyze the lactone but also catalyzes additional rearrangements. The secondary hydroxyl in the A ring of gibberellin A_1 is readily epimerized under these conditions to a more stable isomer (pseudogibberellin A_1) which is biologically inactive (49, 54). Gibberellin A_3 undergoes an allylic rearrangement in alkaline solution to the isomeric lactone formulated by Cross *et al.* as structure VIII, which they had originally assigned to GA-3 itself (50). This isomer is biologically inactive.



VIII

Many other derivatives of the gibberellins have been prepared and some of these have been tested for biological activity (68, 69). In general, it has

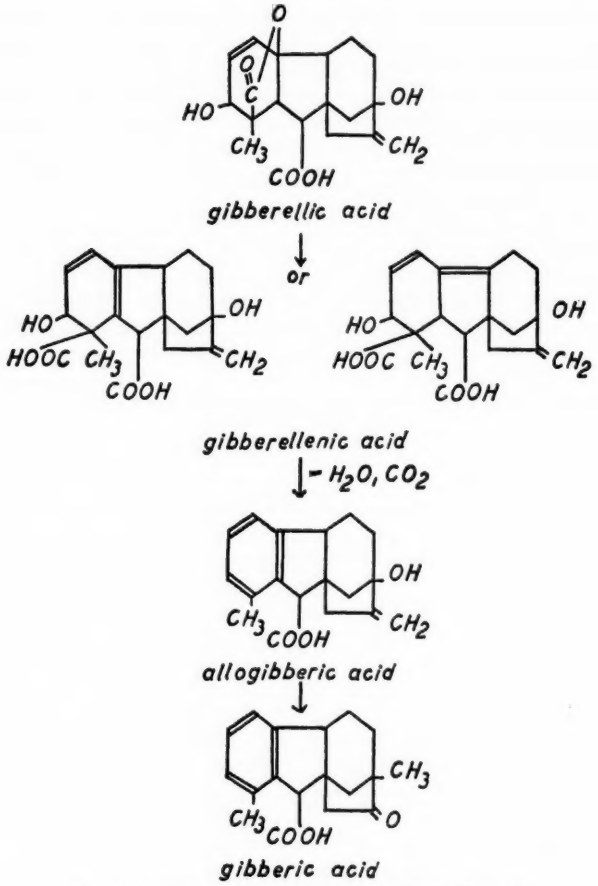


FIG. 1. Acid-catalyzed degradation of gibberellic acid.

been observed that those substances derived from the gibberellins in which a structural modification in the A or B rings has occurred are biologically inactive. The acyl derivatives of the secondary hydroxyl of the A ring are exceptions to this generalization (70). On the other hand, modifications in the C or D rings often lead to active derivatives, although the activities are usually less than those of the parent substance. In this latter category are gibberellin C, dihydrogibberellin A_1, dihydrogibberellin A_4 and a derivative of gibberellin A_4 in which the exocyclic methylene group in ring D is re-

placed by a keto oxygen (obtained on ozonization of gibberellin A_4) (68).

Physico-chemical techniques.—A number of solvent systems have been used for filter paper chromatography of the gibberellins (12, 13, 29, 71, 72, 73). Bioassay of sections of the chromatograms still seems to be the most specific and sensitive means of locating the gibberellins [see (12, 13, 71, 72, 73) for examples], particularly when rather crude mixtures are chromatographed; but this is a relatively tedious and time-consuming procedure. Gibberellin A_3 can be located by means of its characteristic fluorescence after treatment with sulfuric acid (12, 13, 74), but this may tend to be obscured in the presence of other materials on the chromatogram. Other gibberellins do not respond to this test. Other spray reagents have been used (29, 71, 72, 74). Among these, an acidic periodate-permanganate reagent suggested by Mitchell (72) seems to be the most sensitive.

Gibberellin A_4 and GA-5 can be relatively easily resolved from the other gibberellins by paper chromatography (34, 64), but the resolution of mixtures of GA-3, GA-1, and GA-2 is more difficult. Most of the solvent systems referred to above fail to satisfactorily separate these substances. Bird & Pugh (74) report that prolonged development in a benzene-acetic acid-water solvent leads to the separation on filter paper of GA-3 and GA-1. Stodola *et al.* (75) developed a column partition method for the fractionation of GA-3 and GA-1, and Grove *et al.* (44) reported a similar method. Takahashi *et al.* (29) were able to separate the methyl esters of GA-3, GA-1, and GA-2 on alumina columns as did Grove *et al.* (44).

A quantitative fluorimetric assay for gibberellin A_3, relying on the formation of a fluorogen in sulfuric acid solution, has been reported (76). Gibberellenic acid interferes, but this can be removed or corrected for if necessary. The other gibberellins do not respond in this assay. Selected absorption bands in the infrared have been utilized for quantitative estimations of GA-3 and GA-1 in mixtures of the two (78). Infrared spectra of the free acids or their methyl esters have frequently been used in the qualitative identification of isolated gibberellins. [See (10, 26, 29, 32, 44, 64, 77) for infrared spectral characteristics of the pure gibberellins.]

Baumgartner *et al.* have developed a number of useful isotopic labeling methods for the assay of small amounts of gibberellins in relatively crude mixtures (78, 79). One procedure calls for the conversion of the extracted gibberellins to their methyl-C^{14} esters by treatment with diazomethane-C^{14}, resolution on paper chromatograms, and detection and quantitation by radioassay. They have also developed an isotope dilution assay based on recovery of added gibberellin A_3-H^3 of high specific activity. Zweig *et al.* have employed this method for estimation of gibberellin A_3 residues on treated plants (80). Baumgartner *et al.* have also devised a method involving both of these approaches for the estimation of small amounts of gibberellin A_3 in biological materials. Arison *et al.* have described a mass isotope dilution assay procedure utilizing deuteriogibberellin A_3 (81).

Biosynthesis of the gibberellins.—The carbon skeleton of the gibberellins suggests a close biosynthetic relationship to the diterpenes. Cross *et al.* suggested that the isoprenoid nature of their proposed carbon skeleton for gibberellin A_3 was one reason for favoring it over other alternatives (49). Birch *et al.* have outlined an hypothesis for the biosynthesis that involves the condensation of four mevalonate units to an acyclic diterpene which in turn is converted to a tricyclic diterpene (a substituted perhydrophenanthrene derivative) (67, 82). Modification of this intermediate, by contraction of the center ring, elimination of an angular methyl group, and a rearrangement of a type proposed by Wenkert in the biosynthesis of phyllocladene (83) to generate the C-D rings, would lead to the gibberellin carbon skeleton. Reactions analogous to each of these types of modifications can be cited. The general features of this hypothesis are similar to the current proposals for the biosynthesis of sterols and other cyclic isoprenoid compounds.

Birch *et al.* have tested these ideas by allowing cultures of *Fusarium moniliforme* to metabolize mevalonate-2-C^{14} and acetate-1-C^{14} in separate experiments (67, 82). Labeled gibberellin A_3 was isolated and degraded by suitable chemical means to yield information regarding the specific activity of the carbon in each of seven different positions of gibberellin A_3. The pattern of labeling was consistent with that predicted from the above hypothesis. Furthermore, the amount of label in one position as compared with the entire molecule suggested that eight positions were derived specifically from the C-1 of acetate and four positions were derived specifically from the C-2 of mevalonate, findings also consistent with the above hypothesis. Zweig has also shown with C^{14}-labeling studies that the two carbons of acetate are incorporated into gibberellin A_3 by the fungus (84). Thus, the view that the gibberellins are biosynthetic relatives of the diterpenes seems well founded. There is at present, however, no information concerning the chemical nature of intermediates or any details of the biosynthetic mechanisms.

It is assumed that the biosynthesis of gibberellins in flowering plants follows similar pathways to those in the fungus, but there is no evidence at present to document this. The close structural relationships of GA-3, GA-1, and GA-5, and of GA-2 and GA-4, suggests that they might be metabolically interrelated by the following transformations:

$$\text{gibberellin } A_5 \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \text{gibberellin } A_1 \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \text{gibberellin } A_3$$

$$\text{gibberellin } A_4 \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \text{gibberellin } A_2$$

No direct evidence for these reactions has been obtained. Determinations of the relative biological activities of the different gibberellins on dwarf mutants of maize have revealed that GA-5 is as active or more active than GA-3 on four of the mutants (dwarf-2, dwarf-3, dwarf-5, and anther ear-1) but is

less than 10 per cent as active in stimulating the growth of another (dwarf-1) (37). None of the other gibberellins shows such a differential response on these nonallelic, single gene mutants. One interpretation of the results is to assume that gibberellin A_5 is a normal intermediate in the production of a functional growth regulator and dwarf-1 is blocked in its ability to utilize this intermediate, whereas the other dwarf mutants are not.

Bioassays for Gibberellin-like Substances

Cultivars, varieties, and single gene mutants from several species of flowering plants have been used in bioassays to detect the presence of gibberellin-like substances from plant extracts; they have also served to follow biological activity in the isolation and chemical identification of the gibberellins. The species include *Oryza sativa* L. (rice), *Avena sativa* L. (oats), *Triticum vulgare* Vill. (wheat), *Zea mays* L. (maize), *Phaseolus vulgaris* L. (beans), *Pisum sativum* L. (peas), *Pharbitis nil* Chois. (morning glory), *Perilla ocymoides* L., and *Rudbeckia bicolor* Nutt. Activity is measured as a net increase in the length of stem or leaves of intact plants, or portions of these grown in culture. For some of the bioassays, activity is also measured in terms of flowering response. Classification of unknown substances as gibberellin-like is based on a similarity in growth response induced by unknown substances and the chemically known gibberellins; specificity of response is an important criterion in this classification. While certain of the bioassays are known to show some response to chemically known regulators other than the gibberellins, this nonspecificity has been minimized by chromatography of the extracts to remove active fractions which can be associated with the indole auxins or the kinins. Purification of extracts also serves to remove inhibitors which may mask the presence of gibberellin-like substances in the extracts.

Use of intact plants.—The use of seedlings of *Oryza sativa* for bioassay dates to the early work of Kurosawa on the "bakanae" (foolish seedling) disease of *Oryza* (16). Heated sterile filtrates of the fungus *Fusarium moniliforme* are added to test tubes containing sterile sand. From one to three kernels are placed on the sand and allowed to germinate and grow for a period of eight to ten days. Increases in height of treated seedlings over the nontreated controls serve as an indication of biological activity. The sensitivity of the seedling response was shown to be dependent on the cultivar of *Oryza* used for bioassay. The numerous Japanese studies on the production of the active principle from the fungus, on the purification and isolation work which led to the first crystalline gibberellin, and on the more recent studies of the chemistry of the gibberellins have used variations of Kurosawa's assay; such an assay has also been employed by Stodola *et al.* in the isolation of gibberellin-X (31), and by Murakami (85, 86), and Dudman & Nicols (87) to screen plant extracts for the presence of gibberellin-

like substances. Seedlings of the *Oryza* cultivar, Aichi-Asahi, are reported to be sensitive to as little as 0.01 μg. "gibberellin A" per plant, and insensitive to indole auxins and to kinetin (86).

Seedlings of normal *Zea mays* have been used by Nisikado *et al.* (88, 89, 90) to measure the pathogenicity, i.e., "bakanae" effect, of different strains of the fungus, *Fusarium moniliforme*. Normal seedlings have also served as an assay to screen a large number of fungi and actinomyces for the presence of gibberellin-like substances [Curtis (91)]. Since the single gene dwarf mutants d_1, d_2, d_3, d_5, and an_1 are known to be specific and highly sensitive in their growth response to the gibberellins, they have been used in a number of studies for the detection of gibberellin-like substances from plant extracts (11, 12, 34, 37, 84; 92 to 98). The mutants respond to as little as 0.001 μg. gibberellin A_3 per plant; they are apparently insensitive to auxins and to kinins. The material to be assayed is placed in the cup-shaped leaf of mutant seedlings at the time the first leaf blade is beginning to unfold from the coleoptile. Activity is indicated by a net elongation of the first or second leaf sheath over nontreated dwarf controls. At 30°C., maximum response occurs within seven days after treatment; the responses of the mutants have been evaluated statistically for the quantitative estimation of relative activities (98). The response curve, plotted as log response against log dosage, is linear over the range of 0.001 μg. to 10.0 μg. gibberellin A_3 per plant.

Seedlings of the Black Valentine cultivar of *Phaseolus vulgaris* were used to follow the biological activity of preparations obtained from gibberellin producing strains of *Fusarium moniliforme* [Mitchell & Angel (99, 100); Massey & Mitchell (101) cf. (1) for abstract of ref. (101)]. Extracts from the fungus were applied to the bud or to the distal end of the primary leaf petiole; a net increase in length of the shoot indicated activity in the assay. IAA was reported to be inhibitory in their tests. Activity of gibberellins and of gibberellin-like substances has also been measured by seedling elongation of other cultivars of *P. vulgaris* (102, 103). The Black Valentine cultivar was also used by Mitchell *et al.* in 1951 (104) to report evidence for unknown phytohormones from ether diffusates of young seed of *P. vulgaris*. Diffusates were incorporated into lanolin and the lanolin applied unilaterally to the first internode of *Phaseolus* seedlings: curvature of the stem was used as a quantitative assay to measure the amount of extractable hormone in seeds at different stages of development. Recently the data from these curvature assays have been interpreted as evidence for relatively high amounts of gibberellin-like substances in very young seed (2, 4, 36); however, gibberellins are not active in this (93) or other curvature tests (105, 106). Mitchell *et al.* also applied the lanolin preparations as a band around the first internode and recorded marked elongation of the internodes; similarly applied IAA resulted in elongation confined to one internode. These and other biological tests of the unknown phytohormones were probably measuring components of a mixture of native auxins and gibberellin-like substances.

Intact seedlings of dwarf cultivars of *Pisum sativum* have served to iden-

tify substances having properties similar to the gibberellins (36, 71, 107 to 109). Seedlings are treated by leaf or bud application, or by spray, and the increase in shoot length over controls used as a measure of activity. For the cultivar Meteor, the response curve relating log response (per cent) against log dosage of gibberellin A_3 is linear over a 10,000-fold dosage range (108); this assay is sensitive to as little as 0.001 μg. gibberellin A_3 per plant. Adenine is apparently inactive.

Hirono *et al.* (41) have described an assay based on the seedling response of the dwarf Kidochi cultivar of *Pharbitis nil,* a short-day plant. The epicotyls of 3-day-old seedlings are treated with the material to be assayed and net shoot elongation as well as increase in the flowering response used as a measure of gibberellin-like activity. The response curve for shoot elongation is similar to that for *Zea mays* and *Pisum sativum.* The assay is highly specific and sensitive to minute amounts of gibberellin.

The seedling growth of a cultivar of *Perilla ocymoides* has served as a bioassay for the presence of gibberellin-like substances (110), as has shoot elongation and flowering of *Rudbeckia bicolor* (111, 112). Burrow *et al.* (113) used seedlings of *Triticum vulgare* to follow biological activity in their early studies on the chemistry of the gibberellins.

Sections of plants.—The growth response of sections of excised leaves from several grasses has been used as an assay to detect gibberellin-like substances and to follow gibberellin activity (13, 36, 92, 109; 114 to 118). In general the methods are variations of the *Avena* and *Hordeum* leaf section tests originally described by Hayashi & Murakami in 1954 (118), where sections of the young leaf sheath are cultured in sucrose plus the substance to be tested for activity; in this test gibberellin was highly active and IAA either active or inhibitory depending on the origin of the section from the sheath. Sections from the basipetal region were inhibited by IAA and PAA; sections of acropetal origin were stimulated by IAA and PAA. In the leaf section test of *Triticum* described by Radley (36), both kinetin and IAA can be inhibitory. One advantage of leaf section assays is the relatively short period of time (20 to 24 hr.) required for completion of growth of the sections. The nonspecific leaf section assays are useful for initial screening studies in which additional tests for activity are made with more specific bioassays.

Very young excised coleoptiles of *Triticum* have been shown to respond to gibberellin and kinetin but not to IAA (119); they have been used to assay chromatographically purified plant extracts, components of which probably contain gibberellin-like substances.

Gibberellin-like Substances

Distribution and amounts.—Prior to the 1950's the fungal gibberellins were thought to be related only indirectly to the growth of flowering plants; when added to seedlings of *Oryza sativa* they stimulated unusual shoot elongation, similar in appearance to effects of the "bakanae" disease of *Fusarium* infected rice. Gibberellins were also known to induce the same type of shoot

growth in seedlings of a number of other flowering plants. Considerable emphasis was placed on the fact that only certain strains of one species of the fungus, *Fusarium moniliforme,* accumulated these substances. The idea that gibberellins might be naturally occurring in organisms other than the fungus, came from the appearance of certain specific examples of gibberellin-induced growth in flowering plants; the examples involved contrasting growth habits, i.e., cases where both tall and dwarf (or rosette) habits of growth were known within the same species of plant. Addition of gibberellin to the repressed types of growth resulted in shoot elongation such that the treated plants appeared similar, if not identical, to the nontreated tall types (42, 120 to 122). The "normalcy" of these responses would immediately lead one to suspect that the gibberellins might be native plant growth regulators of flowering plants. Their level or availability in the plant could then be responsible for the contrasting types of growth (35, 39). Evidence soon appeared in the literature for the presence of substances from flowering plants having biological properties similar to the gibberellins isolated from the fungus (11, 12, 13, 110, 117). The presence of gibberellin-like substances from the young seed or fruit of *Phascolus vulgaris* L., *Pisum sativum* L., *Lupinus* sp., *Aesculus californica* (Spach.) Nutt., *Echinocystis macrocarpa* Greene, *Zea mays* L., *Persea americana* Mill., *Prunus domestica* L., *P. armeniaca* L., *P. amygdalus* Batsch., and *Nicotiana glauca* Graham was reported by West & Phinney in 1956 (11). Phinney *et al.* in 1957 (12) used the d_1 mutant of *Z. mays* for assay. Activity was obtained from concentrated acetone-water, methanolic, or ether extracts; different R_F values suggested the presence of a family of gibberellin-like substances. Also in 1956, Radley (13) reported gibberellin-like substances from extracts of young shoots of dwarf and tall cultivars of *Pisum sativum;* ethyl alcohol extracts were purified by solvent partition and by paper chromatography and the eluants from the chromatograms tested for activity by an *Avena* leaf section assay. In 1957, Lona (110) reported the presence of similar substances from purified acetonic extracts of the young inflorescences of *Brassica napus* L. cv. Oleifera based on a *Perilla* seedling assay. It was readily apparent that gibberellin-like substances were probably one of the components of the unknown phytohormones of *Phaseolus seed* reported by Mitchell *et al.* in 1951 (104) [ether diffusates of young *Phaseolus* seed were shown to be active in the d_1 *Zea mays* assay, also in the *Oryza* seedling assay (11, 12, 85)]. Following these initial reports, information rapidly accumulated for the widespread occurrence of gibberellin-like substances among flowering plants. Corcoran (93) obtained evidence for such substances from acetonic extracts of the young seed or fruit of 35 species of flowering plants representing the 20 genera: *Acacia, Astragulus, Benincasa, Calendula, Casimiroa, Cassia, Cleome, Coreopsis, Cucumis, Cytisus, Gazania, Helianthus, Lupinus, Passiflora, Phaseolus, Prunus, Retama, Thermopsis, Ulex,* and *Wisteria.* Dwarf mutants of *Zea mays* were used for bioassay. Murakami (85, 86) employed the *Oryza* seedling assay to obtain evidence for gibberellin-like substances from chromatographed extracts obtained from 15 species of Leguminosae repre-

senting the genera: *Albizzia, Arachis, Canavalia, Cercis, Cytisus, Dolichos, Glycine, Maackia, Medicago, Phaseolus, Pisum, Robinia, Sophora, Vigna,* and *Wisteria.* Hirono, Ogawa & Imamura (41) and Ogawa & Imamura (123, 124) have obtained gibberellin-like substances, based on a dwarf *Pharbitis* assay, from 23 species of plants representing the genera: *Arachis, Calonyction, Canavalia, Cassia, Coix, Erigeron, Glycine, Lupinus, Oryza, Pharbitis, Phaseolus, Prunus, Quamoclit, Raphanus, Rudbeckia, Sechium, Vicia, Vigna,* and *Zea.* Nickell (107) used a *Pisum* seedling assay to obtain evidence for gibberellin-like substances from acetonic extracts of tissue cultures from seven species representing the five genera: *Agave, Helianthus, Melilotus, Phaseolus,* and *Vinca.* Several workers have also reported the presence of gibberellin-like substances from members of the genera: *Chrysanthemum, Cocos, Fumaria, Hyoscyamus, Lactuca, Malus, Phaseolus, Pisum, Rudbeckia,* and *Triticum* (71, 92, 95, 96, 97, 103, 108, 114, 115); the evidence is based on chromatographically purified extracts tested on a variety of bioassays. *Fusarium moniliforme* strains which accumulate gibberellin-like substances are of wider host range and geographic distribution than previously reported (2, 94); other fungi than *Fusarium* have been found to contain gibberellin-like substances from assays based on the growth and flowering of *Rudbeckia* (111, 112). It has been suggested that gibberellins may be of universal occurrence in the plant kingdom (93).

Gibberellin-like substances are present in various parts of the plant; such substances have been reported not only from the shoot (13, 36, 37, 71, 95, 96, 108, 114, 115), and the root (58, 107), but also from the leaves (36, 95, 96, 107, 122), the stem (36, 107), seed and seed parts (36, 41, 85, 86, 92, 93, 97, 109, 117, 123, 124), fruit wall (36, 93, 124), and young inflorescences (110, 124). Both young and mature seed (36), and young and mature tissues of stem and leaf (36, 95, 96) are reported to contain these substances. The tissue cultures of Nickell (107), which accumulate gibberellin-like substances, originated from such diverse sources as stem, petiole, root, leaf, and cotyledon; the type of tissue cultures varied from crown gall to virus tumor to callus.

Radley (36), McComb & Carr (108), Corcoran (93), and Hirono *et al.* (41) have estimated amounts of extractable gibberellin-like substances obtained from various parts of flowering plants. Radley's estimates are based on response values matched with equivalent GA-3 responses; McComb & Carr, Hirono *et al.,* and Corcoran converted response values to GA-3 equivalents from a GA-3 dosage response curve. The estimates were made from purified as well as crude extracts of the plant material. Radley obtained activities equivalent to 0.25 μg. GA-3 per gm. fresh weight from chromatographically purified methanolic diffusates of the young seed of *Phaseolus multiflorus,* 0.004 μg. GA-3 per gm. fresh weight from the dry seed of *Triticum vulgare, Vicia faba,* and *Pisum sativum,* and 0.0016 μg. GA-3 per gm. fresh weight from seedling shoots of *Pisum sativum.* Activity from the seed appeared to be equally distributed between the testa, cotyledons, and embryo. Extracts from the immature pods of *Phaseolus multiflorus* contained

gibberellin-like substances in amounts comparable to values for seedling shoots of *Pisum sativum*. McComb and Carr have reported values equivalent to 0.021 μg. GA-3 per gm. fresh weight from extracts of seedlings of *Pisum sativum*. Corcoran has estimated acetonic diffusates from young seed of *Lupinus succulentus* Dougl. to contain 17 GA-3 μg. equivalents per gm. fresh weight for cotyledon epicotyl, 0.6 μg. equivalents per gm. fresh weight for hypocotyls, 0.3 μg. GA-3 equivalents per gm. fresh weight for testae, and 0.6 μg. GA-3 equivalents per gm. fresh weight for hypocotyls; diffusates from the pods of *Lupinus* had an equivalent activity of 0.1 μg. GA-3 per gm. fresh weight. Extracts from *Echinocystis macrocarpa* cotyledons contained the equivalent of 25.0 μg. GA-3 per gm. fresh weight, breis of the endosperm, 500 μg. GA-3 per ml. No activity was obtained from extracts of the fruit wall of *Echinocystis*. Hirono *et al.* estimated ethyl alcohol diffusates from the young fruit of *Prunus persica* Bartsch to contain 1.5 μg. GA-3 equivalents per gm. fresh weight; higher values were obtained from the endosperm of *Prunus persica* and *Lupinus luteus* L. Apparently, rapidly growing tissues contain the highest amounts of gibberellin-like substances.

Physiological Role of Gibberellins

One interesting aspect of gibberellin-induced growth is the implication that the response may be a reflection of the level or availability of native gibberellins in the plant. Positive correlations between amount of native gibberellins and amount of growth might be expected if native gibberellins are one of the factors regulating plant growth. Comparisons are available on the levels of gibberellin-like substances from seedlings of normal and dwarf mutants of *Zea mays* (37), from shoots and seed of normal and dwarf cultivars of *Pisum sativum* (36), from nonelongating and elongating rosetted shoots of *Hyoscyamus niger* (95, 96), *Rudbeckia speciosa* and *Chrysanthemum morifolium* (114, 115); there is also information from three species of flowering plants on changes in amounts of gibberellin-like substances from the seed during the growth of the seedling (93).

Single gene dwarfism.—Gibberellins are known to induce a normal type growth response for a number of single gene dwarf mutants of *Zea mays* (12, 121), *Pisum sativum* (39), *Hordeum sativum* (125), and *Lolium perenne* (126), for certain cultivars (of unknown genotype) of *Pisum sativum* (120), *Vicia faba,* and *Phaseolus vulgaris* (120), *Lathyrus odoratus* L. (127) and *Pharbitis nil* (41), also for certain dwarf segregants from crosses between lines of *Tephrosia vogellii* (128). The response of single gene dwarf mutants to gibberellins has an interesting interpretation based on gene action at the biochemical level (4, 35, 37, 39). Each dwarfing gene may interefere with a specific step in a native gibberellin pathway, the products of which are necessary for normal growth. One of the consequences of such a genetic block could be the absence or presence, in reduced amounts, of native gibberellins in the mutant as compared to the normal; supplying gibberellin to the mutant would raise the gibberellin level to give normal

growth. Also, the kinds of gibberellins rather than their total amounts could be affected through the accumulation of gibberellin intermediates that occupy steps prior to the genetic block (37); addition of gibberellins representing steps after the genetic block would result in normal growth. It has also been suggested that certain examples of dwarfism may be due to the accumulation of inhibitors which are overcome by increasing the gibberellin level of the plant (36, 39). All of the above examples could be expected to occur, since the reasons for dwarfism will vary depending on the particular mutant gene responsible for the dwarf habit of growth (121).

Phinney (37) has found the level of acetone-extractable, gibberellin-like substances to be appreciably less from dwarf mutants of *Zea mays* than from their normal sibs, less than half as much; such substances were obtained from seedling shoots of the mutants d_1 and d_2, while no gibberellin-like substances were obtained from the mutants d_3, d_5, and an_1. Preliminary studies gave no indication for the presence of specific gibberellin inhibitors in the mutants. Radley (36) has also obtained gibberellin-like substances from methanolic extracts of the shoots and seed of tall (Improved Pilot) and dwarf (Meteor) cultivars of *Pisum sativum;* no appreciable differences were found between the two. Radley has suggested that the differences in growth habit could be due to the accumulation of inhibitors by the dwarf cultivar rather than a difference in gibberellin content.

Rosetted biennials and long-day annuals.—Plants having the rosetted habit of growth when maintained in long day or in the absence of a specific cold requirement, can be induced to bolt, i.e., elongate, and flower by treatment with gibberellins (129); normally these plants require either a specific photoinduction or a specific cold treatment. The similarity between the response to light or cold and the gibberellin response would suggest that native gibberellins may be concerned with the mechanisms initiated by day length and/or temperature. Lang *et al.* (130) and Bunsow *et al.* (131) have shown that gibberellin-like substances obtained from flowering plants will replace the light and cold requirement as do the chemically known gibberellins. But even more direct evidence is now available to link the presence of native gibberellins with bolting and flowering. Lang [see (95, 96)] has found a qualitative and quantitative difference in acetone-extractable, gibberellin-like substances between induced and noninduced annual *Hyoscyamus niger.* Purified extracts from the shoots of induced plants that had begun to bolt were found to contain appreciably more gibberellin-like substances than noninduced plants. There was also some evidence for the appearance of a new gibberellin-like substance from induced shoots, which was apparently absent in the extracts obtained from noninduced material. Dwarf mutants of *Zea mays* were used for bioassay. Harada and Nitsch (115), and Nitsch (114) have reported changes in methanolic extractable gibberellin-like substances during the flower initiating processes in the long-day plant *Rudbeckia speciosa,* and the cold-requiring Shuokan cultivar of *Chrysanthemum morifolium*; *Avena* mesocotyl and leaf section tests were

used for bioassay. Chromatographically purified extracts from shoots of noninduced plants of both species were apparently lacking in gibberellin-like substances, while those from induced plants contained appreciable quantities of these materials. The correlations of changes in gibberellin-like substances with bolting support the idea that light and temperature induction, at least in the cases studied, may be mediated through the availability of native gibberellins in the plants. Whether floral initiation is an indirect consequence of bolting rather than a direct gibberellin effect is as yet unresolved; only those long-day plants that have the rosette habit of growth can be induced to bolt and flower with gibberellin treatment.

Seed and fruit growth.—Parthenocarpy resulting from gibberellin treatment has been reported for *Lycopersicum esculentum* Mill. (132), *Cucumis sativus* L., *Solanum Melongena* L., *Capsicum frutescens* L. (5, 133, 134), and *Zephyranthes* sp. (hybrid) (135). The parthenocarpic fruit of *Zephyranthes* were found to contain seed with normally appearing seed coats devoid of embryos. Likewise, unpollinated ovules of *Cooperia pedunculata* Herb., cultured in media containing gibberellins, have been reported to develop normal appearing seed coats without embryos (136). The growth response of the fruit of *Vitis vinifera* L. to gibberellins is most marked among the seedless cultivars (137 to 139). Embryos of *Gossypium hirsutum* L. are accelerated in their growth in the presence of gibberellins (140). Also, tissue cultures obtained from cotyledons of *Vicia faba* (141) and excised mesocarp tissue from *Citrus medica* L. (142) respond to gibberellins. Growing seed are known to be a relatively rich source of gibberellin-like substances, with relatively lower amounts being reported from the pericarp (36, 93). Corcoran has shown an interesting correlation between growth of the seed and amounts of acetone-extractable gibberellin-like substances from the seed for *Echinocystis macrocarpa, Lupinus succulentus,* and *Phaseolus vulgaris* (93). On a per seed basis, lowest amounts were obtained from the very young seed and seed that had nearly reached maturity. Increases in gibberellin-like substances occurred during the most rapid growth of the seed with maximal levels appearing at the time the seed approached maturity. The growth of the pericarp was not positively correlated with changes in gibberellin-like substances in the seed, since fruit growth was virtually completed before appreciable increases in gibberellin-like substances were detected. These various kinds of data would suggest that the developing embryo may be a site of gibberellin production and that these substances are necessary for the development of the seed and possibly the fruit wall.

Interaction Between Gibberellins and Other Growth Regulators

The growth of a plant must depend in part on the interaction of endogenous gibberellins, auxins, and kinins, together with the other, as yet unknown, native substances having regulatory properties. Limiting amounts of any one of these, or more than one, could be the reason for single or multiple requirements of induced growth. Information on changes in

amounts of endogenous gibberellin and auxin has been reported for *Rudbeckia speciosa* and *Chrysanthemum morifolium* (93, 94) where increases in both auxin and gibberellin-like substances are correlated with the onset of bolting and flowering following light or temperature induction. More extensive evidence is available on the requirement of exogenous gibberellin and IAA, and also exogenous gibberellin and kinetin, in the stimulation of certain types of plant growth. Asprey *et al.* (143) have shown the elongation of the isolated sporophyte of *Pellia epiphylla* to be dependent on the addition of both gibberellin and IAA. The growth response is apparently more than additive; the presence of the thallus substitutes for the IAA requirement. Kuse (144) has found the elongation of debladed petioles of *Ipomoea batatas* cv. Norin No. 1. to be dependent on both gibberellin and IAA; the growth response is less than additive and the IAA requirement can be replaced by the presence of the leaf blade. Wareing (145) has reported an interesting case of synergism between gibberellin and IAA in cambial activity for certain types of woody dicotyledonous plants. Gibberellin treatment results in the production of a zone of new tissue consisting of unlignified cells; IAA alone results in the production of a narrow zone of new xylem vessels that are lignified. The two together result in a marked stimulation of cambial activity with the formation of lignified xylem elements. A number of studies have reported either additive or synergistic growth responses to gibberellin plus IAA for stem sections of *Pisum sativum* (146 to 154). Synergism between IAA and gibberellin in promoting extension of *Triticum* leaf sections has also been observed (36). Explant cultures of citron fruit show greater callus formation in the presence of both gibberellin and IAA than with either alone (142), and embryos of *Gossypium hirsutum* exhibit optimal growth in the presence of both of these growth regulators (140). Kuraishi & Hashimoto (155) have found kinetin and gibberellin to be additive for the increase in fresh weight of leaf discs, less than additive for the expansion of leaf discs of *Raphanus sativus*, L. cv. Riso Kaikon. Skinner *et al.* (156) report synergism between gibberellin and kinetin for seed germination of cultivars of *Axonopus compressus* Beauv., *Trifolium repens* L. and *Lactuca sativa* L. Vasil (157) has shown tetrad formation in excised anthers of *Allium cepa* L. to be similar to intact controls when the excised anthers are cultured in White's media supplemented with both gibberellic acid and kinetin. Depressed growth responses due to kinetin are overcome in some cases with gibberellin treatment (158, 159).

Suggestions have been made for the action of gibberellins through an auxin-mediated mechanism (148 to 151; 160 to 162), based on examples of interaction between gibberellin and auxin, also on examples of decreases in *in vivo* IAA-oxidase activity following gibberellin treatment. Purves & Hillman (153) and Hillman & Purves (154) feel that the available evidence does not justify this interpretation. There may be more reason to consider gibberellins as one of a number of native regulators all of which are re-

sponsible for the growth and differentiation of a plant. While the genotype or combination of genotype and specific environment may result in a growth response dependent on the addition of a single substance, the implication remains for the necessity of interaction of numerous growth regulators within the plant for this growth response. The chemical studies by Skoog *et al.* (163) and Steward *et al.* (14) have placed emphasis on the control of growth through the interaction of numerous regulators rather than a single auxin-mediated mechanism.

LITERATURE CITED

1. Stodola, F. H., *Source Book on Gibberellin, 1828–1957* (Agr. Research Service, U.S.D.A., Peoria, Ill., 1958)
2. Stowe, B. B., and Yamaki, T., *Ann. Rev. Plant Physiol.*, **8,** 181–216 (1957)
3. Stowe, B. B., and Yamaki, T., *Science,* **129,** 807–16 (1959)
4. Brian, P. W., *Biol. Revs., Cambridge Phil. Soc.* **34,** 37–84 (1959)
5. Wittwer, S. H., and Bukovac, M. J., *Econ. Botany,* **12,** 213–55 (1958)
6. Went, F. W., *Rec. trav. Botan. Neerl.,* **25,** 1–116 (1928)
7. Bentley, J. A., *Ann. Rev. Plant Physiol.,* **9,** 47–80 (1958)
8. Kogel, F., Haagen-Smit, A. J., and Erxleben, H., *Z. physiol. Chem.,* **235,** 201–16 (1935)
9. Bukovac, M. J., and Wittwer, S. H., in *Preceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in Press)
10. MacMillan, J., Seaton, J. C., and Suter, P. J., *Proc. Chem. Soc.,* 325–26 (1959)
11. West, C. A., and Phinney, B. O., *Plant Physiol.* (Suppl. 31), **xx** (1956)
12. Phinney, B. O., West, C. A., Ritzel, M., and Neely, P. M., *Proc. Natl. Acad. Sci. U.S.,* **43,** 398–404 (1957)
13. Radley, M., *Nature,* **178,** 1070–71 (1956)
14. Steward, F. C., and Shantz, E. M., *Ann. Rev. Plant Physiol.,* **10,** 379–404 (1959)
15. Sawada, K., *Formosan Agr. Rev.,* **36,** 10, 16 (1912)
16. Kurosawa, E., *Trans. Natl. Hist. Soc. Formosa,* **16,** 213–27 (1926)
17. Kurosawa, E., *Trans. Natl. Hist. Soc. Formosa,* **20,** 218–39 (1930)
18. Kurosawa, E., *Ann. Phytopath. Soc. Japan,* **4,** 65–66 (1934)
19. Ito, S., and Shimada, S., *Ann. Phytopath. Soc. Japan,* **2,** 322–38 (1931)
20. Ito, S., and Shimada, S., *Ann. Phytopath. Soc. Japan,* **2,** 471 (1932)
21. Shimada, S., *Kagaku (Toyko),* **3,** 53–54 (1933)
22. Shimada, S., *Ann. Phytopath. Soc. Japan,* **3,** 69 (1934)
23. Yabuta, T., Kambe, K., and Hayashi, T., *Nippon Nôgei-kagaku Kaishi,* **10,** 1059–68 (1934)
24. Yabuta, T., *Agr. and Hort.,* **10,** 17–22 (1935)
25. Yabuta, T., and Sumiki, Y., *Nippon Nôgei-kagaku Kaishi,* **14,** 1526 (1938)
26. Cross, B. E., *J. Chem. Soc.* 4670–76 (1954)
27. Brian, P. W., Grove, J. F., Hemming, H. G., Mulholland, T. P. C., and Radley, M., *Plant Physiol.,* **33,** 329–33 (1958)
28. Stodola, F. H., *Symposium Lecture Am. Inst. Biol. Sci.* (Storrs, Conn., 1956)
29. Takahashi, N., Kitamura, H., Kawarada, A., Seta, Y., Takai, M., Tamura, S., and Sumiki Y., *Bull. Agr. Chem. Soc. Japan,* **19,** 267–77 (1955)

30. Curtis, P. J., and Cross, B. E., *Chem. & Ind. (London)*, 1066 (1954)
31. Stodola, F. H., Raper, K. B., Fennell, D. I., Conway, H. F., Sohns, V. E., Langford, C. T., and Jackson, R. W., *Arch. Biochem. Biophys.*, **54**, 240–45 (1955)
32. Takahashi, N., Seta, Y., Kitamura, H., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **21**, 396–98 (1957)
33. Takahashi, N., Seta, Y., Kitamura, H., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **23**, 405–7 (1959)
34. West, C. A., in *Proceedings of the Fourth International Conference on Plant Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
35. Phinney, B. O., and West, C. A., *Proc. Intern. Genet. Symposium, 1956*, (Cytol. Suppl.), 384–385 (1957)
36. Radley, M., *Ann. Botany (London)*, **22**, 297–307 (1958)
37. Phinney, B. O., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
38. Emerson, R. A., Beadle, G. W., and Fraser, *N.Y. State Agr. Exp. Sta. Mem. No. 39*, 1–83 (1935)
39. Brian, P. W., *S.E.B. Symposium on Biological Action of Growth Substances*, 166–82 (Cambridge University Press, New York, N.Y., 1957)
40. Lamprecht, H., *Agr. Hortique Genetica*, **5**, 16 (1947)
41. Hirono, Y., Ogawa, Y., and Imamura, S., *J. Plant and Cell Physiology* (Tokyo), (In press)
42. Lang, A., *Naturwissenschaften*, **43**, 284–85 (1956)
43. Melchers, G., *Biol. Zentr.*, **57**, 568–614 (1937)
44. Grove, J. F., Jeffs, P. W., and Mulholland, T. P. C., *J. Chem. Soc.*, 1236–40 (1958)
45. Cross, B. E., Grove, J. F., MacMillan, J., and Mulholland, T. P. C., *J. Chem. Soc.*, 2520–36 (1958)
46. Morrison, A., and Mulholland, T. P. C., *J. Chem. Soc.*, 2536–39 (1958)
47. Mulholland, T. P. C., *J. Chem. Soc.*, 2693–2701 (1958)
48. Morrison, A., and Mulholland, T. P. C., *J. Chem. Soc.*, 2702–5 (1958)
49. Cross, B. E., Grove, J. F., MacMillan, J., and Mulholland, T. P. C., *Proc. Chem. Soc.*, 221–22 (1958)
50. Cross, B. E., Grove, J. F., MacMillan, J., Moffatt, J. S., Mulholland, T. P. C., and Seaton, J. C., *Proc. Chem. Soc.*, 302–3 (1959)
51. Cross, B. E., Grove, J. F., McCloskey, P., and Mulholland, T. P. C., *Chem. & Ind. (London)*, 1345–46 (1959)
52. Kitamura, H., Seta, Y., Takahashi, N., Kawarada, A., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **21**, 71–72 (1957)
53. Seta, Y., Kitamura, H., Takahashi, N., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **21**, 73–74 (1957)
54. Takahashi, N., Seta, Y., Kitamura, H., Kawarada, A., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **21**, 75–76 (1957)
55. Takahashi, N., Seta, Y., Kitamura, H., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **21**, 327–28 (1957)
56. Seta, Y., Takahashi, N., Kitamura, H., Takai, M., Tamura, S., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **22**, 61–62 (1958)
57. Seta, Y., Takahashi, N., Kitamura, H., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **22**, 429–31 (1958)

58. Takahashi, N., Seta, Y., Kitamura, H., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan,* **22,** 432–33 (1958)
59. Kitamura, H., Takahashi, N., Seta, Y., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan,* **22,** 434–35 (1958)
60. Kitamura, H., Takahashi, N., Seta, Y., Kawarada, A., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan,* **23,** 344–46 (1959)
61. Stork, G., and Newman, H., *J. Am. Chem. Soc.,* **81,** 5518 (1959)
62. Stork, G., and Newman, H., *J. Am. Chem. Soc.,* **81,** 3168 (1959)
63. MacMillan, J., and Suter, P. J., *Naturwissenschaften,* **45,** 46–47 (1958)
64. West, C. A., and Phinney, B. O., *J. Am. Chem. Soc.,* **81,** 2424–27 (1959)
65. Kawarada, A., and Sumiki, Y., *Bull. Agr. Chem. Soc. Japan,* **23,** 343–44 (1959)
66. Gerzon, K., Bird, H. L., and Woolf, D. O., *Experientia,* **13,** 487–89 (1957)
67. Birch, A. J., Richards, R. W., and Smith, H., *Proc. Chem. Soc.,* 192–93 (1958)
68. Sumiki, Y., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
69. Bukovac, M. J., and Wittwer, S. H., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R. Ed., Iowa State College Press, Ames, Iowa, in press)
70. Imperial Chemical Industries, Ltd., *Pat. Appl. Case Q11504A.11656A* (Commonwealth of Australia, 28.6.55. 10190, 1955)
71. Simpson, G. M. *Nature,* **182,** 528–29 (1958)
72. Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists,* **41,** 182–85 (1958)
73. Smale, B. C., and Daly, E. J., *J. Agr. Food Chem.,* **6,** 751–52 (1958)
74. Bird, H. L., and Pugh, C. T., *Plant Physiol.,* **33,** 45–46 (1958)
75. Stodola, F. H., Nelson, G. E. N., and Spence, D. J., *Arch. Biochem. Biophys.,* **66,** 438–43 (1957)
76. Kavanaugh, G., and Kuzel, N. R., *J. Agr. Food Chem.,* **6,** 459–65 (1958)
77. Washburn, W. H., Scheske, F. A., and Schenck, J. R., *J. Agr. Food Chem.,* **7,** 420–22 (1959)
78. Baumgartner, W. E., Lazer, L. S., Dalziel, A. M., Cardinal, E. V., and Varner, E. L., *J. Agr. Food Chem.,* **7,** 422–25 (1959)
79. Baumgartner, W. E., *Proc. Symposium Advances in Tracer Applications of Tritium,* 33–37 (New York, N.Y., October 1958)
80. Zweig, G., and Cosens, G. R., *J. Agr. Food Chem.,* **7,** 717–19 (1959)
81. Arison, B. H., Speth, O. C., and Trenner, N. R., *Anal. Chem.* **30,** 1083–85 (1958)
82. Birch, A. J., and Smith, H., *Biosynthesis of Terpenes and Sterols,* 245–66 (Little, Brown and Co., Boston, Mass., 1959)
83. Wenkert, E., *Chem. & Ind. (London),* 282–84 (1955)
84. Zweig, G., DeVay, J. E., and Cosens, G. R., *Plant Physiol.* (Suppl. 33), xxxviii (1958)
85. Murakami, Y., *Botan. Mag. (Tokyo),* **70,** 376–82 (1957)
86. Murakami, Y., *Botan. Mag. (Tokyo),* **72,** 36–43 (1959)
87. Dudman, W. F., and Nichols, R., *Nature,* **183,** 899–900 (1959)
88. Nisikado, Y., and Matsumoto, H., *Tottori Nôgaku-Kwaihô.,* **4,** 200–211 (1933)
89. Nisikado, Y., Matsumoto, H., and Yamuti, K., *Ber. Ôhara Inst. landwirtsch. Forsch. Kurashiki, Japan,* **6,** 113–30 (1933)
90. Nisikado, Y., Matsumoto, H., and Yamuti, K., *Ber. Ôhara Inst. landwirtsch. Forsch. Kurashiki, Japan,* **6,** 131–47 (1933)

91. Curtis, R. W., *Science,* **125,** 646 (1957)
92. Nitsch J, P., *Bull. soc. botan. France,* **105,** 479–82 (1958)
93. Corcoran, M. R. (Doctoral thesis, Univ. of California, Los Angeles, Calif., 1959)
94. Gordon, W. A., *Nature* (In press)
95. Lang, A., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna,* 1958, 126–40 (1959)
96. Lang, A., *Planta* (In press)
97. Blumenthal-Goldschmidt, S., and Lang, A., *Nature* (In press)
98. Neely, P. M. (Doctoral thesis, Univ. of California, Los Angeles, Calif., 1959)
99. Mitchell, J. E., and Angel, C. R., *Phytopathology,* **40,** 872–73 (1950)
100. Mitchell, J. E., and Angel, C. R., *Phytopathology,* **41,** 26–27 (1951)
101. Massey, L. M., and Mitchell, J. E., *Am. Inst. Biol. Sci.* (Ithaca, N.Y., 1952)
102. *Gibberellins, Tech. Bull.* (Merck & Co., Rahway, N.J., 1957)
103. Manzelli, M. A. (Personal communication, 1958)
104. Mitchell, J. W., Skaggs, D. P., and Anderson, W. P., *Science,* **114,** 159–61 (1951)
105. Sumiki, Y., *Nippon Nôgei-kagaku kaishi,* **26,** 393–97 (1952)
106. Kato, J., *Mem. Coll. Agr., Kyoto Univ. Chem. Ser.,* **20,** 189–93 (1953)
107. Nickell, L. G., *Science,* **128,** 88–89 (1958)
108. McComb, A. J., and Carr, D. J., *Nature,* **181,** 1548–49 (1958)
109. Radley, M., and Dear, E., *Nature,* **182,** 1098 (1958)
110. Lona, F., *Ateneo parmense,* **28,** 111–15 (1957)
111. Krasilnikov, N. A., Chailakhyan, M. Kh., Aseeva, I. V., and Khlopenkova, L. P., *Doklady Akad. Nauk. S.S.S.R.,* **123 11,** 1124–27 (1958)
112. Krasilnikov, N. A., *Priroda,* **47,** 81–84 (1958)
113. Borrow, A., Brian, P. W., Chester, V. E., Curtis, P. J., Hemming, H. G., Henehan, C., Jeffreys, E. G., Lloyd, P. B., Nixon, I. S., Norris, G. L. F., and Radley, M., *J. Sci. Food Agr.,* **6,** 340–48 (1955)
114. Nitsch, J. P., *Proc. Intern. Congr. Biochem., 4th Congr., Vienna, 1958,* 126–40 (1959)
115. Harada, H., and Nitsch, J. P., *Plant Physiol.,* **34,** 409–15 (1959)
116. van Overbeek, J., Racusen, D. W., Tagami, M., and Hughes, W. J., *Plant Physiol.* (Suppl. 32), xxxii (1958)
117. Murakami, Y., *Botan. Mag. (Tokyo),* **69,** 258–62 (1956)
118. Hayashi, T., and Murakami, Y., *Nippon Nôgei-kagaku Kaishi,* **28,** 543–45 (1954)
119. Ricard, J. R., and Nitsch, J. P., *Compt. rend.,* **247,** 1891–93 (1958)
120. Brian, P. W., and Hemming, H. G., *Physiol. Plantarum,* **8,** 669–81 (1955)
121. Phinney, B. O., *Proc. Natl. Acad. Sci., U.S.,* **42,** 185–89 (1956)
122. Lang, A., *Naturwissenschaften,* **43,** 257–58 (1956)
123. Ogawa, Y., and Imamura, S., *Proc. Japan Acad.,* **34,** 631–32 (1958)
124. Ogawa, Y., and Imamura, S., *Botan. Mag. (Tokyo)* (In press)
125. Stoy, V., and Hagberg, A., *Hereditas,* **44,** 516–22 (1958)
126. Cooper, J. P., *New Phytologist,* **57,** 235–38 (1958)
127. Brian, P. W., Hemming, H. G., and Lowe, D., *Physiol. Plantarum,* **12,** 15–29 (1959)
128. Irvine, J. E., and Ruben, H. F., *Nature,* **185,** 115 (1960)
129. Lang, A., *Publ. Am. Assoc. Advance. Sci.,* No. 55, 329–50 (1959)

130. Lang, A., Sandoval, J. A., and Bedri, A., *Proc. Natl. Acad. Sci., U.S.*, **43**, 960–64 (1957)
131. Bunsow, R., Penner, J., and Harder, R., *Naturwissenschaften*, **45**, 46–47 (1958)
132. Wittwer, S. H., Bukovac, M. J., Sell, H. M., and Weller, L. E., *Plant Physiol.*, **32**, 39–41 (1957)
133. Wittwer, S. H., and Bukovac, M. J., *Mich. State Univ. Agr. Expt. Sta. Quart. Bull.*, **39**, 1–28 (1957)
134. Persson, A., and Rappaport, L., *Science*, **127**, 816 (1958)
135. Sacher, R. C., and Kapoor, M., *Plant Physiol.*, **34**, 168–71 (1959)
136. Sacher, R. C., and Kapoor, M., *Naturwissenschaften*, **45**, 552–53 (1958)
137. Weaver, R. J., *Nature*, **181**, 851–52 (1958)
138. Weaver, R. J., and McCune, S. B., *Hilgardia*, **28**, 297–350 (1959)
139. Stewart, W. S., Ching, F. T., and Halsey, D. D., *Proc. Am. Soc. Hort. Sci.*, **72**, 120–26 (1959)
140. Dure, L. S., and Jensen, W. A., *Botan. Gaz.*, **118**, 254–61 (1957)
141. Nickell, L. G., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
142. Schroeder, C. A., and Spector, C., *Science*, **126**, 701–702 (1957)
143. Asprey, G. F., Benson-Evans, K., and Lyon, A. G., *Nature*, **181**, 1351 (1958)
144. Kuse, G., *Botan. Mag. (Tokyo)*, **71**, 151–59 (1958)
145. Wareing, P. F., *Nature*, **181**, 1744–45 (1958)
146. Kato, J., *Mem. Coll. Sci. Univ. Kyoto, Ser. B*, **20**, 189–93 (1953)
147. Kato, J., *Physiol. Plantarum*, **11**, 10–15 (1958)
148. Brian, P. W., and Hemming, H. G., *Nature*, **179**, 417 (1957)
149. Brian, P. W., and Hemming, H. G., *Ann. Botany (London)*, **22**, 1–17 (1958)
150. Brian, P. W., in *Proceeding of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
151. Galston, A. W., and Warburg, H., *Plant Physiol.*, **34**, 16–22 (1959)
152. Purves, W. K., and Hillman, W. S., *Physiol. Plantarum*, **11**, 29–35 (1958)
153. Purves, W. K., and Hillman, W. S., *Physiol. Plantarum*, **13**, (In press)
154. Hillman, W. S., and Purves, W. K., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
155. Kuraishi, S., and Hashimoto, T., *Botan. Mag. (Tokyo)*, **70**, 86–92 (1957)
156. Skinner, C. G., Talbert, F. D., and Shive, W., *Plant Physiol.* **33**, 190–94 (1958)
157. Vasil, I. K., *Science*, **126**, 1294–95 (1957)
158. Humphries, E. C., *Ann. Appl. Biol.*, **46**, 346–51 (1958)
159. Humphries, E. C., *Nature*, **181**, 1081–82 (1958)
160. Pilet, P. E., *Compt. rend.*, **245**, 1327–28 (1957)
161. Pilet, P. E., and Murgler, W., *Schweiz. Botan. Ges. Ber.*, Berne, Switzerland, **68**, 54–63 (1958)
162. Galston, A. W., in *Proceedings of the Fourth International Conference on Plant Growth Regulation* (Klein, R., Ed., Iowa State College Press, Ames, Iowa, in press)
163. Skoog, F., and Miller, C. O., *Soc. Exptl. Biol. (Great Britain) Symposium, Biological Action of Growth Substances, 11th Symposium*, 118–31 (1957)

AUTHOR INDEX

A

Aabye Jensen, E., 342, 346, 347, 349, 350, 352, 354, 355, 356, 358
Aach, H. G., 220, 278
Abe, S., 329, 330, 333, 334, 335, 336
Åberg, B., 239
Abolina, G., 196
Abrahams, M. D., 81, 82, 86, 95
Abrahamson, E. W., 383
Acerbo, S. N., 67
Ada, G. L., 281
Adams, E., 58
Adamson, D., 246
Adamson, H., 246
Adler, E., 68, 70
Afanasieva, T. P., 181
Agar, H. D., 11
Ahmed, M. K., 155, 156
Aitken, R. A., 315
Akabori, H., 329
Akai, S., 304, 311, 312, 316
Akazawa, T., 74
Akulovich, N. A., 368, 369
Albaum, H. G., 258
Albersheim, P., 135, 247, 300
Albrecht, F., 114, 115, 116
Alexander, H. E., 280
Alexander, M., 26
Al Kholy, A. A., 355
Allen, E. K., 25, 26, 28
Allen, M. B., 27, 37, 38, 39, 40, 41, 42, 50, 393, 394, 395, 396, 399
Allen, O. N., 25, 26, 28, 155
Allen, P. J., 335
Allen, T. C., 259
Allfrey, V., 10
Allington, W. B., 286
Allison, R. M., 31
Al-Rawi, S., 277
Alsac, N., 119
Amer, F. A., 115
Amesz, J., 394
Amman, A., 316
Anagnostopoulos, P. T., 213
Andel, O. M. van, 146
Anderer, F. A., 278
Anderson, A. J., 28
Anderson, D. G., 97
Anderson, H. W., 155
Anderson, S. G., 281
Anderson, W. P., 155, 424, 426
Anderssen, F. G., 147, 148, 149
Andersson, D. Q., 346, 355
Andreae, S. R., 261
Andreae, W. A., 242, 245, 246, 263
Andreeva, I. N., 146, 148, 150
Andreeva, T. F., 397
Andresen, N., 331
Angel, C. R., 424
Angot, M., 346, 349
Ansevin, A. T., 278
Applewhite, T. H., 252
Arison, B. H., 421
Arisz, W. H., 127, 129, 141, 142, 146, 252
Armarego, W. L. F., 264
Arnold, W., 19, 373, 376,
Arnold, W. A., 374, 375
Arnon, D. I., 18, 27, 37, 38, 39, 40, 41, 42, 43, 44, 50, 51, 52, 393, 394, 395, 396
Aronoff, S., 170, 171, 177, 183
Arrigoni, O., 259, 260
Arthur, J. M., 120, 123, 204
Arthur, W. E., 373
Asai, G. N., 173
Aseeva, I. V., 425, 427
Ashkinazi, M. S., 380
Ashworth, P. R., 312
Asprey, G. F., 264, 431
Atkins, W. R. G., 147, 341, 343, 344, 345, 351, 354
Atkinson, D. E., 283
Audus, L. J., 239, 251, 254
Austin, T. S., 343, 344, 352
Avadhani, P. N., 87, 90
Avakjan, A. A., 195, 203
Avery, G. S., Jr., 257
Avron, M., 37, 38, 43, 44, 45, 50, 395
Axelrod, B., 96
Axelrod, J., 68
Ayengar, P. K., 62

B

Bach, M. K., 29, 30, 33
Baker, E., 300
Baker, H., 146
Baker, M., 342
Baker, R. S., 242, 253, 262, 264
Bakhuyzen, H. L. van de Sande, 216
Bald, J. G., 286, 287
Baldwin, R. E., 155, 156
Ball, C. D., 67, 247, 308, 312
Ball, N. G., 184
Bandurski, R. S., 96, 97, 98, 239, 252, 257, 260, 262
Bang, K. D., 236
Bange, G. G. J., 114, 115
Bannister, T. T., 378, 381
Barber, D. A., 127-40
Barber, H. M., 208
Barker, J. W., 251
Barltrop, J. A., 42
Barrier, G. E., 173, 175, 176
Barton, L. V., 225
Bassarskaja, M. A., 220
Bassett, E. W., 59
Bassham, J. A., 43, 47, 48, 397
Bate-Smith, E. C., 64, 67
Bathurst, N. O., 149
Battersby, A. R., 75
Bauer, G., 356
Baumgartner, A., 115
Baumgartner, W. E., 421
Bawden, F. C., 277, 280, 283, 286, 288, 289, 290
Bayley, S. T., 247, 249, 251, 257, 300
Bayliss, N. S., 33
Bazhanova, N. V., 371
Beadle, G. W., 414
Beale, H. P., 286, 287
Beans, H. W., 1, 11
Bechi, A., 192, 197, 222
Becker, R. S., 373
Beckman, C., 303, 313, 316
Bedri, A., 429
Beer, M., 168, 300, 310
Beevers, H., 83, 85, 92, 93, 96, 103, 257
Beklenishev, C. V., 358
Belavtseva, E. M., 370
Belford, D. S., 299, 315
Belikov, I. F., 177
Beljaev, N. V., 206
Bell, C. E. Z., see ZoBell, C. E.
Bell, G. D. H., 197, 199
Bell, L. N., 388
Bell, T. A., 309, 311
Benda, G. T. A., 288
Bendall, D. S., 100
Bendix, S., 207
Benitez, A., 365, 366

Bennet-Clark, T. A., 81, 83, 84, 87, 92, 94, 251, 252, 263, 264, 308, 312
Bennett, C. W., 277, 293, 294
Bennett, H. S., 8
Bennett, J. P., 147, 148, 149
Bennett, S. H., 156
Benson, A. A., 106, 151
Benson-Evans, K., 264, 431
Bentley, J. A., 239, 412
Berge, G., 346, 349, 352
Berger, J., 257
Berger-Landefeldt, U., 112
Bergersen, F. J., 28
Bergmann, L., 285
Bergold, G., 277
Bergström, H., 117
Berker, H. A., 28
Bernard, F., 344, 358
Beth, K., 241, 243
Beyrich, H. B., see Breckheimer-Beyrich, H.
Bharucha, F. R., 81, 86, 87
Biddulph, O., 151, 158, 159, 160, 161, 171, 175, 178, 179, 183, 184
Biddulph, S., 178, 179
Biddulph, S. F., 158, 159, 160
Billek, G., 67, 68, 69
Biochenko, E. A., 392
Birch, A. J., 61, 419, 422
Bird, H. L., 419, 421
Bishop, C. T., 247, 249, 300
Bishop, O. N., 128, 129
Bitcover, E. H., 153
Blaauw, A. H., 193
Blaauw, O. H., 14, 19
Black, F. L., 284
Blackman, F. F., 138
Blackwood, A. C., 62
Blake, J., 213
Blanchard, F. A., 155, 156
Bland, D. E., 68, 71
Blaney, L. T., 222
Blinks, L. R., 17, 19, 368
Bliss, L. C., 120
Blum, G., 250
Blum, R., 315
Blumenthal-Goldschmidt, S., 424, 427
Blundell, M. J., 71
Boardman, N. K., 292, 293
Bocchi, A., 196, 222
Boedtker, H., 277, 279
Bogen, H. J., 251, 254
Bogorad, L., 71, 72
Bogorov, B. G., 352, 358
Bogoyavlenskii, A. N., 345, 353
Böhni, E., 303, 304, 311, 312
Boichenko, E. A., 397
Bois, E., 146
Bolas, B., 206
Bollard, E. G., 141-66; 147, 148, 149, 150, 151, 160, 179
Bond, G., 25, 26, 31, 136, 149
Bonde, E. K., 208
Bonin, J. F., see Favre-Bonin, J.
Bonner, J., 6, 7, 9, 10, 21, 83, 100, 135, 141, 239, 242, 243, 245, 246, 247, 248, 249, 251, 252, 253, 254, 256, 257, 258, 260, 262, 264, 312, 333
Bonner, W. D., 83
Bonner, W. D., Jr., 32, 242, 256, 258, 260
Booij, H. L., 253
Bornstein, B. T., 28
Boroughs, H., 249
Borrow, A., 425
Borthwick, H. A., 206
Bottelier, H., 336
Bottelier, H. P., 255
Bourdon, J., 48
Bowler, E., 300
Boyle, L. M., 286
Boysen-Jensen, P., 249
Braarud, T., 342, 344, 357
Brachet, J., 5, 10
Bradbeer, C., 103
Bradbeer, J. W., 82, 93, 96, 97, 99, 100, 101, 105
Bradfield, J. G., 11, 12, 14
Bradley, D. F., 49
Brady, L. R., 74
Brambring, F., 116
Brandt, K., 354
Braun, H. J., 167
Brauner, L., 250, 252
Braunitzer, G., 278
Breazeale, E. L., 145
Breazeale, J. F., 145
Breckheimer-Beyrich, H., 324
Brenchley, G. H., 314
Brian, P. W., 155, 156, 240, 264, 265, 411, 412, 413, 414, 419, 424, 425, 426, 428, 429, 431
Brian, R. C., 253
Briggs, G. E., 3, 135, 142
Brill, C., 364
Brin, G. P., 370, 378, 379, 381, 383, 389, 390, 391, 392, 393, 394, 396
Brodie, H. W., 160
Brody, M., 368
Brody, S. S., 368, 370, 372
Brooks, F. T., 314
Brouardel, J., 346, 347, 350
Brouwer, R., 127, 128, 131, 133, 134, 143
Brovchenko, M. I., 171
Brown, A. H., 106
Brown, H. T., 111, 113, 114, 116
Brown, J. C., 152, 153, 154
Brown, J. M. A., 81, 85, 86, 90, 91, 92, 98, 104
Brown, J. S., 369
Brown, J. W., 155, 156, 242
Brown, M. E., 26
Brown, S. A., 59, 65, 66, 67, 68, 71, 73
Brown, W., 303, 308, 310
Broyer, T. C., 127, 128, 129, 130, 133, 134, 137, 142, 144, 146, 160, 178
Brucker, W., 62
Bruinsma, J., 81, 82, 86, 92
Bruns, F. H., 64
Bryan, W. H., 248, 308, 312
Bryant, A. E., 136
Bubnov, N. N., 386
Buch, K., 345
Buchtela, K., 69, 70, 71
Budagowski, A. I., 116
Buffel, K., 247, 253, 308, 312
Bukovac, M. J., 175, 204, 220, 411, 412, 419, 430
Bulen, W. A., 31
Bumpus, D. F., 343, 357, 358
Bungenberg de Jong, H. G., 253
Bunsow, R., 429
Burr, G. O., 160
Burrell, R. C., 100, 105
Burris, R. H., 28, 29, 30, 31, 33, 34, 99, 100, 105, 259
Burström, H., 146, 147, 148, 246, 247, 252, 253
Busse, M., 246, 249
Butenko, R. G., 177
Butler, C. G., 112
Butler, G. W., 135
Butsko, S. S., 377
Butterfass, Th., 179
Büttner, K., 111, 115
Butts, J. S., 242
Buvat, R., 4, 5, 6
Byerrum, R. U., 67
Byers, H. G., 143
Byrde, R. J. W., 311
Bystrova, M. I., 366

C

Caldwell, J., 177, 286
Calhoun, B., 175

Calvert, A., 196, 206
Calvet, F., 292
Calvin, M., 42, 47, 48, 49, 60, 96, 103, 106, 373, 374, 376, 398, 400
Camp, W. G., 331
Campbell, C. W., 220
Canning, R. E., 142
Canny, M. J., 264
Caplin, S. M., 241
Cardinal, E. V., 421
Carlier, A., 247, 308, 312
Carnahan, J. E., 28
Carr, D. J., 222, 263, 425, 427
Carsner, E., 199
Carter, C. E., 328
Carvell, K. L., 155
Casper, D. L. D., 278
Casperson, G., 116, 117, 119, 123
Caspersson, T., 10
Casterman, C. H., see Hamers-Casterman, C.
Castle, J. E., 28
Cathey, H. M., 214
Cederstrand, C., 368
Ceithaml, J., 97
Cepikova, A., 208, 211
Ceponis, M. J., 303, 312
Cesnokov, V., 199
Chailakhyan, M. Kh., 171, 177, 425, 427
Chakravarti, S. C., 206, 221, 222, 237
Chalmers, R., 368
Chambers, R., 7
Chamova, K. G., 392
Cahmpéroux, A., 195, 222
Champigny, M. L., 100, 104, 105, 106
Chan, A. P., 214
Chan, H. T., 203
Chance, B., 393
Chantrenne, H., 293
Chao, Fu-Chuan, 9
Charles, A., 157
Chayen, J., 5, 310
Cheadle, V. I., 167, 174, 181, 184, 185, 186
Chen, S. L., 179
Cheniae, G., 32
Cheo, P. C., 279
Chestakov, V. E., 220
Chester, K. S., 283
Chester, V. E., 425
Chidester, J. L., 292
Ching, F. T., 430
Chinoy, J. J., 197, 237, 260
Chmora, S. N., 116, 388
Chodat, P., 236
Cholodny, N. G., 220
Chona, B. L., 304
Chouard, P., 191-238; 193, 195, 202, 203, 204, 205, 208, 211, 212, 213, 214, 215, 217, 219, 221, 222, 224, 225, 237
Christensen, H. N., 254
Christian, J. E., 100
Christiansen, G. S., 249, 253, 256, 257, 258
Chroboczek, E., 199, 205
Chubb, W. O., 146
Church, A. B., 367
Claes, H., 192, 197
Clark, J. E., 263, 264
Clark, P., 7
Clark, T. A. B., see Bennet-Clark, T. A.
Clarke, I. D., 100
Claude, A., 4, 5
Cleland, R., 242, 246, 247, 312
Clendenning, K. A., 392
Clenokova, I. V., 236, 237
Clifford, P. A., 155
Cline, J. F., 128
Clor, M. A., 264
Closs, R. L., 119
Coblentz-Mishke, O. Y., 347, 352
Cochrane, G. W., 292
Cohen, A. L., 331
Cohen, D., 264
Cohen, M., 294
Cohn, W. E., 328
Cole, J. S., 304, 306, 311
Cole, M., 306, 307, 311, 317
Coleman, J. W., 387
Coleman, M. T., 97
Coles, C. H., 89, 97, 100
Collander, R., 128
Collie, J. N., 61
Collins, R. P., 303, 313, 314
Colmano, G., 369, 370
Colvin, J. R., 300
Commoner, B., 250, 252, 254, 256, 257, 258, 260, 263, 280, 281, 292, 376, 385
Comte, P., 72
Conn, E. E., 11, 59, 73, 74, 97
Connard, M. H., 178
Conrat, H. F., see Fraenkel-Conrat, H.
Conway, H. F., 413, 423
Cooil, B. J., 258, 260, 312
Cooper, F. P., 300
Cooper, J. P., 211, 428
Cooper, J. R., 60
Cooper, L. H. N., 345, 351, 354
Corcoran, M. R., 424, 426, 427, 428, 430, 431
Corden, M. E., 312
Corey, R. A., 155
Corlett, J., 349, 350
Corwin, N., 349, 355
Cory, R., 158, 159, 160, 171, 175, 178, 179, 183, 184
Cosens, G. R., 421, 422, 424
Cowan, I. R., 121
Cowling, E. B., 315, 318
Cox, S. F., 264
Coyne, B. A., 254
Crafts, A. S., 129, 142, 146, 155, 156, 169, 172, 173, 176, 184, 186
Cross, B. E., 413, 414, 416, 417, 419, 421
Crowdy, S. H., 154, 155, 156, 157
Cruzado, H. J., 155, 156
Currie, R. J., 343, 347, 352
Currier, H. B., 167, 174, 181, 184, 186, 251, 264
Curtis, D. S., 203
Curtis, O. F., 112, 173, 180
Curtis, P. J., 413, 425
Curtis, R. W., 424
Cushing, D. H., 343, 349, 350, 358
Cutkamp, L. K., 155
Czailachjan, M. C., 196, 206, 237
Czaja, A. T., 250

D

Dain, B. J., 377, 380
Dainty, J., 135
Dalberg, L. Y., 241, 242, 261, 266
Dalleux, G., 331
Dalton, A. J., 9
Daly, E. J., 421
Dalziel, A. M., 421
Danielli, J. F., 3
Danielson, C. E., 8
Dao-The-Touan, 197
Dardart, J., 105, 106
Das, N. K., 261, 262
David, R., 193, 197, 206, 207, 220
David, W. A. L., 155
Davies, E. B., 27
Davis, B. D., 56, 57, 58, 59, 63, 64, 77
Davis, D. E., 155
Davson, H., 3
Dawson, R. F., 146, 148, 151
Dear, E., 425, 427
de Duve, C., see Duve, C. de
Deese, D. C., 261
deHoop, H., see Hoop, H. de
de Jong, H. G. B., see Bungenberg de Jong, H. G.
Delbrück, M., 226

Delwiche, C. C., 26
Demain, A. L., 300, 302
Demkovskii, P. I., 220
Denffer, D. von, 197
De Preter, C. V. S., see Van-Sumere-De Preter, C.
der Hart, J. M. van, see Hart, J. M. van der
De Ropp, R. S., 192, 194, 196, 223, 253, 268
DeRose, H. R., 155, 156
DeStevens, G., 68
De Stigter, H. C. M., 182
Deuel, H., 135, 300, 302
De Vay, J. E., 422, 424
Devine, R. L., 1, 11
Dewey, L. J., 67
Dickson, A. G., 177
Die, J. van, 146, 148, 150
Dietrich, G., 357
Digby, P. S. B., 343
Diksit, N. N., 205
Diller, V. M., 155
Dilung, I. I., 377
Dimbleby, G. W., 146, 148
Dimond, A. E., 141, 303, 305, 306, 312, 313, 314, 316, 318
Dixon, H. H., 147, 184
Dixon, M., 97
Dmitrievsky, O. D., 372, 383
Doesburg, J. J., 312
Doorenbos, J., 86, 192, 193, 204, 206, 213, 214, 236, 237
Dorman, S. C., 155
Dorrenstein, R., 364
Doty, M. S., 343, 344, 346, 349, 350, 352, 356
Doty, P., 279
Doughty, C. C., 31
Douglas, H. C., 11
Drawert, H., 168
Drimmel, J., 122
Dorzdova, N. N., 380
DuBuy, H. G., 256
Dudman, W. F., 423
Dugal, L. C., 146
Dugdale, R., 26
Dugdale, V., 26
Duggar, B. M., 281
Dugger, W. M., Jr., 174, 175, 183, 258
Dulbecco, R., 290
Dulova, V. I., 380
Duloy, M., 13
Duncan, C. W., 315
Dunitz, I. D., 292
Duodoroff, M., 33
Dupéron, R., 195, 219, 220
Dure, L. S., 264, 430
Duve, C. de, 9, 12
Duysens, L. N. M., 14, 19, 42, 47, 364, 387, 388, 394
Dye, M. H., 155, 157

E

Eaton, F. M., 146, 148
Eberhardt, G., 59
Echandi, E., 303, 306, 311, 312, 316
Eckert, E., 111, 113, 114
Eddy, B. P., 315
Edelman, J., 81, 86, 88
Edgington, L. V., 312
Edmondson, W. T., 356
Efeikin, A. K., 195
Egami, F., 29
Eggleston, L. V., 93, 99
Eggman, L., 333, 334
Eguchi, T., 205
Ehrenberg, M., 100
Ehrensvärd, G., 58
Eichel, B., 258
Elliot, W. H., 33
Elliott, R. F., 367
Elsden, S. R., 77
El-Shishiny, E. D. H., 171
Emerson, R., 19, 368, 389
Emerson, R. A., 414
Emerson, R. B., 155, 156
Emerson, R. L., 395
Emswiller, S. L., 206
Engelbrecht, L., 146, 150, 169, 183, 184
Engler, R., 287, 290, 292
Eppley, R., 254
Epstein, E., 127, 134, 135
Erdtman, H., 61, 70, 72
Eremenko, V. T., 194
Erickson, L. C., 170
Erk, S., 113
Ermolaev, V. L., 372, 375, 376
Ermolenko, I. N., 372
Erxleben, H., 412
Esau, K., 158, 167, 174, 181, 184, 185, 186
Eschrich, W., 168
Escombe, F., 111, 113, 114, 116
Esposito, R. G., 27
Etchells, J. L., 309, 311
Evans, H., 81
Evans, H. J.. 27, 32
Evans, K. B., see Benson-Evans, K.
Evstigneev, V. B., 378, 379, 380, 381, 382, 383, 384, 385, 391
Eymers, J. G., 255
Eyster, C., 27
Eyster, H. C., 248, 258

F

Fahraeus, G., 261, 315
Faigle, H., 69
Falk, H., 119
Fang, S. C., 155, 156, 242
Farrant, J. L., 11, 12
Favre-Bonin, J., 72
Fawcett, C. H., 154
Fedorov, V. D., 365
Fedosov, M. V., 345, 358
Fejér, D., 148, 151
Fennell, D. I., 413, 423
Fensom, D. S., 186
Feodorov, A. K., 195
Feofanova, N. D., 197
Feokanova, N. D., 195
Fergus, C. L., 303, 304, 313
Fernando, M., 304, 312
Ferrell, W. K., 160
Ferri, M. G., 155, 156
Fetzmann, E. L., 325
Fidler, J. C., 91
Fiedler, L., 64
Fields, M., 242
Filippenko, I. A., 194, 220
Filippovich, I. I., 220
Findlay, G. H., 146
Fineman, J. F., see Fogelstrom-Fineman, J.
Fischer, A., 168
Fischer, H., 178
Fitton Jackson, S., 5
Fleming, R. H., 358
Flemion, F., 237
Flokstra, J. H., 67
Fogel, S., 250, 252
Fogelstrom-Fineman, J., 400
Fogg, G. E., 25, 346
Folbert, M. B., 400
Ford, C. V., 289, 294
Forrest, L. A., 146, 148
Forti, G., 257, 259
Foster, R. J., 262, 263
Fowden, L., 141, 150
Fox, D. L., 342
Fraenkel, G. S., 55
Fraenkel-Conrat, H., 278, 279, 280, 281, 289, 292
Franck, J., 372, 373, 376, 389
Frank, J., 48
Frankenberger, E., 115, 122
Franklin, R. E., 277, 278
Fraser, 414
Freed, V. H., 155, 156
Frei, E., 185
Freksa, H. F., see Friedrich-Freksa, H.
French, C. S., 364, 366, 367, 368, 369, 378, 389
French, R. C., 257
Frenkel, A. W., 18, 29, 40, 41, 43, 392, 394, 395
Fresco, J. R., 279
Freudenberg, K., 68, 69, 70, 71
Frey-Wyssling, A., 2, 3, 8, 11, 14, 15, 17, 168, 185, 251, 299, 325
Friedberg, M., 197
Friedman, B. A., 303, 312

Friedrich-Freksa, H., 283
Friend, D. J., 195
Friesen, B. S., 279
Frost, P., 248, 261
Füchtbauer, W., 40
Fujimori, E., 373, 374, 383
Fujomoto, Y., 277
Fukui, H. N., 201, 220
Fukuto, T. R., 155, 157
Fulham, E. F., 4, 5
Fuller, R. C., 398
Funderburk, H. H., 155
Funk, H. B., 28
Fushtey, S. G., 310, 311
Füsser, K., 115

G

Gaarder, T., 341, 346, 347
Gaffron, H., 394
Gallup, A. H., 260
Galston, A. W., 239-76; 241, 242, 244, 245, 253, 255, 258, 260, 261, 262, 263, 264, 265, 266, 431
Gamborg, O. L., 59, 66, 72
Gander, J. E., 74
Gardiner, B. O. C., 155
Gardner, D., 155
Gardner, F. P., 211
Gardner, I. C., 31
Garnjobsts, L., 58
Gassner, G., 191, 195, 197
Gauch, H. G., 174, 175, 183
Gäumann, E., 303, 304, 311, 312
Gavrilova, V. A., 378, 379, 380, 381, 382, 383, 384, 385, 391
Gee, M., 306
Geiger, R., 112, 113, 115, 116, 122, 123
Geissman, T. A., 55, 61, 67, 72
Gering, R. K., 31
Gerloff, C. G., 27
Gerretsen, F. C., 27
Gerzon, K., 419
Gessner, F., 349
Gest, H., 34
Ghosh, J. J., 58
Gibbs, M., 96
Gierer, A., 277, 278, 280, 282, 285, 293
Gillbricht, M., 343
Gilligan, W., 315, 316
Ginoza, W., 278, 280, 281, 283, 289, 290
Ginter, W. D., 81, 82, 84
Ginzburg, B. Z., 264, 300, 309
Gish, D. T., 278, 279
Glasziou, K. T., 248, 254, 308, 312
Glover, J., 213
Glover, L. C., 155
Godard, D., 10, 12, 13
Godnev, T. N., 366, 368, 369, 371
Goebel, Christa, 197
Goedherr, J. C., 364, 372, 377, 388
Goering, J., 26
Goldacre, P. L., 258
Goldberg, E. D., 342
Goldenberg, A. B., 367
Goldschmidt, S. B., see Blumenthal-Goldschmidt, S.
Goldstein, L., 292
Gollub, M., 97
Golstein, M., 99
Good, N., 393
Good, N. E., 242, 245, 246, 263
Goodall, D. W., 206
Goodard, D. R., 259
Goodchild, D. J., 289
Goodman, M., 49
Goodwin, R. H., 241
Goodwin, T. W., 62
Gordon, S. A., 239, 245, 262, 263
Gordon, W. A., 424, 427, 431
Gorgy, A., 343, 344, 345
Gorham, P. R., 173, 174, 177, 183
Gothoskar, S. S., 303, 313
Goto, M., 316
Gott, M. B., 194, 216
Gowen, J. W., 282
Graham, H. W., 343
Gran, H. H., 341, 344, 346, 347, 357
Granick, S., 14
Gravilova, M. F., 195
Greathouse, G. A., 315
Green, D. E., 10, 13
Green, M., 308
Greenridge, K. N. H., 145
Greenslade, R. M., 155, 157
Greenwood, A. D., 251
Gregg, M., 304, 312
Gregory, F. G., 81, 83, 86, 87, 88, 92, 192, 193, 194, 195, 196, 216, 219, 220, 221, 223, 226
Greiner, C. M., 97
Griep, W., 122
Grif, V. G., 237
Grigg, G. W., 16, 19
Grimes, R. M., 315
Gripenberg, S., 345
Grisebach, H., 61, 62, 72
Gröber, H., 133
Grøntved, J., 342, 344, 347, 351, 355
Grosclaude, C., 303, 304
Gross, J., 9
Gross, S. R., 58, 59
Grossenbacher, K. A., 146
Grossman, F., 311
Grove, J. F., 155, 156, 413, 414, 416, 417, 419, 421, 422
Grover, R., 260
Grube, K. H., 37, 49
Gruen, H., 241
Gruzinsky, V. V., 372
Gubbenet, E. R., 371
Guernsey, F. S., 207, 221, 260
Guillard, R. R. L., 349
Guminskaya, M. A., 371
Gunsalus, I. C., 60
Günther, G., 194, 199
Gupta, S. C., 304, 306, 309
Gurinovich, T. P., 372
Gustafson, F. G., 175
Guthrie, J. D., 286, 287
Guttenberg, H. von, 136, 254

H

Haagen-Smit, A. J., 412
Hackett, D. P., 10, 11, 12, 13, 32, 250, 252, 256, 257, 258, 260
Hagberg, A., 428
Haidri, S. Z. A., 106
Hakkaart, F. A., 199
Halevy, A., 122
Hall, C. E., 277
Hall, W. E., 155
Halldal, P., 344
Halsey, D. D., 430
Hama, M. S. T., see Tamiko-oh-Hama, M. S.
Hamers-Casterman, C., 290
Hamilton, P. B., 32
Hammer, K. C., 300
Hämmerling, J., 243
Hamner, C. L., 247, 308, 312
Hanaoka, T., 342, 343
Hand, M. E., 253, 262
Hannapel, R. J., 144
Hansch, C., 239, 260, 263
Hänsel, H., 193, 195, 196
Hansen, O. H., see Holm-Hansen, O.
Hansen, V. K., 347, 349, 350, 355, 356, 358
Hanson, J. B., 4, 5, 7, 11, 12, 19, 254, 257, 258
Hanson, T., 330
Harada, H., 425, 427, 428, 429
Harary, I., 97
Harder, R., 429
Harper, B. J. T., 75
Harrington, J. F., 201, 236, 237

Harris, J. L., 277, 278
Harrison, B. D., 286, 289, 292
Hart, J. M. van der, 364, 388
Hart, R. G., 279
Hart, T. J., 344, 345, 353
Härtel, O., 114, 115, 116
Hartig, T., 169, 178
Hartley, G. S., 155, 157
Hartmann, H. T., 213
Hartmann, T. A., 204
Hartmann, W., 236, 237
Hartt, C. E., 160
Hartwich, W., 312
Harvey, C. C., 308
Harvey, H. W., 354, 358
Harvill, E. K., 204
Haschemyer, R., 279
Hash, J. H., 315
Hashimoto, T., 431
Haskins, C. P., 27
Hasman, M., 250, 252, 254
Hassid, W. Z., 174
Hatano, S., 328, 334, 335, 337
Hatcher, E. S. J., 196, 199, 220, 223
Hathway, D. E., 311
Haun, J. R., 155, 156
Haupt, W., 207, 208
Hauser, E. W., 155
Hawkins, G. A., 113, 114
Hay, J. R., 173, 175, 176
Hayaishi, O., 62
Hayashi, T., 264, 309, 324, 327, 412, 425
Hayes, P. M., 42
Hazelkorn, R., 279
Heath, O. V. S., 263, 264
Heinonen, S., 153
Heise, J. J., 376
Heitner-Wirguin, C., 264
Heitz, E., 19, 20
Helder, R. J., 146, 252
Hemaidan, N., 153
Hemming, H. G., 155, 156, 264, 413, 414, 419, 425, 426, 428, 431
Henderson, J. H. M., 261
Hendrich, W., 381
Henehan, C., 425
Henglein, F. A., 300, 312
Henninger, J., 211
Henrici, M., 117
Hensen, V., 341
Hentschel, E., 342, 343, 344, 347, 352, 354, 355
Herschberg, L. H., see Heymann-Herschberg, L.
Hertel, W., 114
Herzog, F., 119
Hewitt, E. J., 27
Heymann-Herschberg, L., 160
Heyn, A. N. J., 242, 246
Hiai, S., 28
Hiatt, H. H., 99
Hibbert, H., 64
Highkin, H. R., 196, 207, 221
Higinbotham, N., 242, 248, 249, 254
Higuchi, T., 59, 64, 68, 70, 71
Hildebrandt, A. C., 285
Hill, G. P., 171, 172, 173, 178, 184
Hill, R., 18, 392, 393, 395
Hillman, W. S., 241, 242, 253, 264, 265, 431
Hino, S., 26, 28
Hinreiner, E., 55
Hinton, C. L., 300
Hirsch, G., 116, 120, 121
Hirono, Y., 414, 425, 427, 428
Hitchcock, A. E., 155, 156
Hoagland, D. R., 127, 128, 130, 131, 133, 134, 137, 142, 144, 149, 158
Hoagland, M. B., 285
Hobbs, M. O., 393, 394
Hoch, G. E., 33, 34
Hodge, A. J., 1, 4, 5, 6, 7, 9, 14, 16, 17, 18, 19, 20, 21
Hofbauer, G., 69
Hoffmann, H., 16, 19
Hofler, K., 7
Hofman, T., 29
Hofmann, G., 111, 113, 114, 115, 116, 118, 119, 120, 121, 122
Hogeboom, G. H., 9
Holch, A. E., 119
Holden, M., 309
Holgate, K. C., 146
Hollaender, A., 281
Holmes, F. O., 282, 286, 288
Holmes, K. C., 277
Holmes, R. S., 152, 153, 154
Holmes, R. W., 343, 344, 352
Holm-Hansen, O., 27, 398, 400
Holt, A. S., 364, 369, 370, 379, 387
Honert, T. H. van den, 127, 131
Hood, K. J., 201
Hoop, H. de, 27
Hooymans, J. J. M., 127, 131
Hope, A. B., 5, 12, 13, 14, 16, 17, 18, 135
Hoppert, C. A., 315
Horecker, B. L., 60, 99
Horwitz, L., 183
Howard, F. D., 11
Howard, F. L., 331
Huang, H. A., 367
Huber, B., 111, 112, 113, 114, 116, 117, 119, 169, 173, 182
Hubert, B., 17
Hudson, W., 283
Hudson, W. R., 294
Huff, E. L., 9
Huggins, W. C., 146, 148
Hughes, W. J., 425
Huiskamp, W. J., 364, 388
Hummel, K., 116
Humphreys, T. E., 175, 258
Humphries, E. C., 431
Hurwitz, J., 58
Husain, A., 303, 311, 313, 314, 316, 317, 318
Hussey, G., 220
Hutchinson, A., 62, 72
Hutner, S. H., 27
Huxley, H. E., 330
Hylmö, B., 127, 132, 133, 134, 135, 143

I

Icha, R., 260
Ichimura, S., 344, 349, 350, 352
Iglinsky, W., 155
Ihida, M., 148
Ihnen, E. D., 394
Imamura, S., 414, 425, 427, 428
Imaseki, I., 75
Inglis, S. D., 248, 308, 312
Ingraham, V. M., 279
Ingram, M., 315
Innes, R. F., 121
Inoue, Y., 207
Irvine, J. E., 428
Ishii, M., 292
Ito, S., 412
Ivanov, L. A., 122
Ivanov, S. M., 207
Ivy, E. E., 155

J

Jablonski, J. R., 261
Jackson, R. W., 413, 423
Jackson, S. F., see Fitton Jackson, S.
Jackson, W. A., 97
Jacobs, E. E., 364, 369, 370
Jacobs, W. P., 248
Jacobson, L., 128, 129, 144, 153
Jagendorf, A. T., 37, 38, 39, 43, 44, 45, 50, 395
Jahn, A., 331
Jahn, T. L., 330
Jahnel, H., 182

Jakob, M., 113, 114
James, W. O., 129, 146
Jang, R., 135, 247, 248
Jansen, E. F., 135, 247, 248, 300
Jarosch, R., 324, 325, 326, 330
Jarvis, W. R., 304, 311, 312
Jedlinski, H., 286
Jeener, R., 290, 292
Jeffreys, E. G., 425
Jeffs, P. W., 414, 421
Jenkins, P. G., 343, 344
Jensen, E. A., see Aabye Jensen, E.
Jensen, H. L., 25, 26, 27
Jensen, P. B., see Boysen-Jensen, P.
Jensen, V., 26
Jensen, W. A., 248, 261, 264, 430
Jerlov, N. G., 343, 356
Jermyn, M. A., 315
Jitts, H. R., 346, 349
Johnson, F. D., 160
Johnson, G. T., 331, 336
Johnson, G. V., 160, 178
Johnson, J., 282
Johnson, L. P. V., 146
Johnson, M. J., 34
Johnson, M. P., 243
Jolchine, G., 82, 93, 99, 100, 101, 103, 104, 105, 106
Jones, D. R., 155, 156
Jones, G. B., 160
Jones, K. M., 81
Jong, H. G. B. de, see Bungenberg de Jong, H. G.
Jordanov, I. T., 203
Jorma, J., 32
Joshi, G. V., 81, 86, 87
Joslyn, M. A., 259, 311
Jost, M., 376
Jouanneau, P., 204
Junges, W., 201, 206, 207

K

Kaars Sijpestein, A., 262
Kagawa, A., 201
Kaji, A., 309
Kala, H., 148
Kaler, V. L., 371
Kalle, K., 345, 357
Kamal, M., 304, 311, 313
Kambe, K., 412
Kamen, M., 393
Kamen, M. D., 47
Kamerbeek, G. A., 242
Kamiya, N., 323-40; 323, 325, 327, 331, 332, 333, 334, 335, 336
Kandler, O., 37-54; 37, 40, 41, 48, 49, 50, 371, 395
Kaper, J. M., 280
Kapoor, M., 430
Karpovich, I. A., 376
Karyakin, A. V., 383
Kasha, M., 373
Kassanis, B., 285, 286, 287, 290, 292
Katagiri, M., 57
Kating, H., 50
Kato, J., 264, 424, 431
Katsumi, M., 264
Katz, E., 364, 365
Kaur, R., 244, 255, 265
Kavanagh, F., 241
Kavanaugh, G., 421
Kawarada, A., 413, 414, 416, 419, 421
Keeler, R. F., 27
Keilin, D., 31, 32
Keller, E. B., 9
Keller, V. P., 135
Kelman, A., 303, 313, 314, 316, 317, 318
Kelso, J. M., 336
Kendall, W. A., 180
Kephart, J., 264
Keresztes, I. M., see Molnár-Keresztes, I.
Kerr, T., 247, 299
Kertesz, Z. I., 247, 300, 311
Kessler, E., 400
Kessler, G., 168
Ketchum, B. H., 349, 355
Ketellapper, H. J., 253
Khlopenkova, L. P., 425, 427
Kholy, A. A. A., see Al Kholy, A. A.
Kieser, M. E., 311
Kil'djuševskij, I. D., 117
King, J. E., 343, 344, 352
King, J. W., 253, 262
King, K. W., 315
Kirkpatrick, H. C., 286
Kisaki, T., 148, 151
Kishimoto, U., 325, 329
Kitamura, H., 413, 416, 417, 419, 421, 427
Kleczkowski, A., 286, 287, 288, 290
Klein, E., 69
Klem, A., 357
Klemmer, H. W., 155
Kleshnin, A. F., 116
Klippart, J. H., 191
Kloen, D., 205
Klug, A., 277, 278
Klug, R. J., 316
Klybas, V., 60
Knight, C. A., 277, 278, 279, 280, 283, 284
Koch, G., 280
Koenigsberger, F., 50
Koepsell, H. J., 34
Kogel, F., 412
Kohl, H. C., Jr., 236, 237
Kohler, E., 292
Kohlmeyer, J., 316
Kohne, L. V., see Vogt-Kohne, L.
Kok, B., 40, 387
Kokin, A. J., 236, 237
Kolesnikov, P. A., 398
Koljo, B., 116, 117
Kollmann, R., 168
Komen, J. G., 364
Kondrat'ev, K. Ia., 116
Kondratyeva, E. N., 365
Konev, S. V., 376
Kőnig, H., 378, 380
Konis, E., 119
Konishi, M., 242
Konovalov, I. N., 196, 207
Kooiman, P., 315
Koontz, H., 158, 159, 160, 175, 178, 179
Korey, S. R., 97
Kornberg, A., 97
Kornberg, H. L., 100, 101, 103
Kossobutskaya, L. M., 364, 366, 367, 368, 369, 376, 387
Kosteckii, T., 199
Kostjucenko, I. A., 195
Kosuge, T., 59, 73
Kozlianinov, M. V., 347, 349
Kramer, P. J., 127, 134, 135, 142, 143, 144, 146, 148, 251, 252
Krasilnikov, N. A., 425, 427
Krasnoseljskaja-Maximova, T. A., 196, 203
Krasnovsky, A. A., 363-410; 364, 365, 366, 367, 368, 369, 370, 371, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 387, 388, 389, 390, 391, 392, 393, 394, 396
Kratzl, K., 68, 69, 70, 71
Kravtsov, L. A., 372
Krebs, H. A., 31, 93, 99, 100, 101, 103
Kreger, D. R., 299
Kremers, R. E., 64, 67, 68
Kreps, E., 344, 345, 351
Kretschmer, A. E., 160
Krey, J., 343, 344
Krippahl, G., 48, 389, 394
Krog, J., 120
Krogh, A., 146, 148
Kromhout, R., 370
Krotkov, G., 87
Kruzilin, A. C., 236, 237
Kruzilin, A. S., 196, 199, 203, 204, 206, 221

Kugler, I., 200
Kuhn, H., 372
Kulaeva, O. N., 146, 148, 150, 179
Kunii, K., 117
Kunitake, G. M., 88, 97, 99, 100, 101, 102, 104, 105, 106
Kunkel, G., 120
Kupke, D. W , 366
Kuraishi, S., 431
Kuroda, K., 323, 325, 327, 330
Kurosawa, E., 412, 423
Kursanov, A. L., 72, 146, 148, 150, 171, 177, 179, 181, 185
Kuse, G., 264, 431
Kutáček, M., 260
Kutyurin, V. M., 371, 398, 399, 400
Kuzel, N. R., 421
Kuznetzova, I. C., 196
Kylin, A., 127, 132, 135

L

Ladefoged, K., 146, 148
Laibach, F., 200
Laird, E. F., Jr., 286
Lakshminarayanan, K., 303, 313
Lamb, J., 311
Lambertz, P., 173
Lamprecht, H., 414
Landefeldt, U. B., see Berger-Landefeldt, U.
Lane, G. A., 400
Lang, A., 88, 192, 193, 197, 198, 199, 204, 216, 221, 222, 225, 237, 414, 424, 426, 427, 428, 429
Lang, H. M., 39
Lang, N. M., 393
Lange, O. L., 117
Langeland, W. E., 248
Langford, C. T., 413, 423
Langston, R., 160
Lapwood, D. H., 305, 312
Lareau, J., 99
Larose, E., 197
Larrieu, C., 212, 223, 225
Larsen, H., 365
Latarjet, R., 287
Laties, G. G., 12, 13, 127, 129, 257, 258
Latimer, H., 254
Latimer, P., 366, 387
Latzko, E., 40
Laudi, G., 259
Lauffer, M. A., 278, 283, 286
Laurencot, H. J., 59
Lauritzen, C. W., 146
Lazer, L. S., 421
Lé, K. N., 208, 237
Leaf, G., 25, 31
Leavenworth, C. S., 81, 82, 84, 86, 95
Lebour, M. V., 354
Ledingham, G. A., 314, 318
Lees, H., 29
Leete, E., 74, 75
Lemoine, P., 292
Leonard, C. D., 153, 154
Leonard, F., 62
Leonard, O. A., 176
Leonhardt, L., 99
Leopold, A. C., 207, 220, 221, 239, 256, 257, 260
Lessard, M., 146
Levin, A. P., 28
Levinson, H. S., 315, 316, 317
Levinthal, C., 284
Levitt, J., 135, 250, 251, 252
Lewis, G. N., 373
Lewis, W. H., 331
Leyon, H., 14, 19, 20
Liakhnovich, J. P., 366
Lieberman, H., 99, 100
Lindner, R. C., 286
Linet, N., 5, 10
Lineweaver, H., 300
Linkola, H., 32
Linnasalmi, A., 32
Linsbauer, K., 324
Linschitz, H., 378, 383, 385
Lippicott, B. B., 376
Lippincott, J. A., 280
Lipskaya, G. A., 371
Litt, M., 279
Little, H. N., 34
Little, J. E., 260
Littlefield, J. W., 9
Litvin, F. F., 365, 366, 367, 368, 371, 374, 375, 387, 394
Liverman, J. L., 242
Livingston, R., 373, 383, 390
Ljunggren, H., 315
Lloyd, P. B., 425
Lockhart, J. A., 261
Löeffler, J. E., 366
Loewenberg, J. R., 151
Loewy, A. G., 325, 333, 335
Loftfield, R B., 293
Lohmann, H., 342, 343, 344, 352, 354
Lojkin, M., 194, 197
Lona, F., 196, 206, 222, 237, 425, 426, 427
Long, W. G., 175
Loomis, W. E., 173, 175, 176, 208, 211
Lorenz, O. A., 184
Loring, H. S., 277, 290
Loughman, B. C., 129, 131
Loustalot, A. J., 155, 156
Lowe, D., 428
Lowry, M. W., 146, 148
Lucas, C. E., 358
Luckwill, L. C., 264
Ludwig, R. A., 313
Lund, H. A., 4, 5, 7, 11, 12, 19
Lundbom, S., 28
Lundegårdh, H., 127, 128, 129, 133, 135, 142, 146, 257
Lyakhnovich, J P., 371
Lynch, V., 373, 387
Lynch, V. H., 97, 99, 100, 101, 105, 378
Lyndon, R. F., 93, 99
Lynen, F., 50
Lyon, A. G., 264, 431
Lysenko, T. D., 191, 193, 194, 195, 197, 215, 216, 222
Lyubomudrov, E. B., 383

M

Maass, H., 311
McCalla, D. R., 59, 65, 66, 67
Maclay, H. K., 376
McCloskey, P., 414, 417
McColloch, R. J., 311
McComb, A. J., 222, 425, 427
McCready, R. M., 300, 306
McCune, D. C., 264
McCune, S. B., 430
McDonnell, K., 314
McGeorge, W. T., 145
McKee, R., 207
McKinney, H. H., 194, 197, 286
McLaren, A. D., 281, 290
McLean, J. D., 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21
MacMillan, J., 240, 412, 413, 414, 416, 418, 419, 421, 422
McRae, D. H., 262
Mac Vicar, R., 255
Mäde, A., 119
Magee, W. E., 29
Maizel, J. V., 151
Makiyama, M., 116
Mangenot, G., 331
Manolova, M. D., 116
Manskaja, S M., 68
Manske, R. H. F., 75
Manzelli, M A., 424, 427
Mapson, L. W., 259
March, R. B., 155, 157
Margara, J., 199, 223
Marion, L., 74
Markle, J., 159
Markley, E., 253
Marré, E., 257, 259, 260, 263

Marsh, P. B., 316
Marshak, A., 292
Marshall, N., 344
Marshall, P. T., 349
Martin, E. M., 7, 9
Martin, G. W., 331
Martin, H., 155
Martin, R. P., 128, 129
Martin-Smith, C. A., 300
Martius, C., 99
Marty, S. G., 207
Marvin, J. W., 146
Maskell, E. J., 147
Maslova, T. G., 369
Mason, D. T., 199, 214
Mason, T. G., 127, 141, 147, 180
Massey, L. M., 424
Massini, P., 106, 155
Massy-Westrop, R. A., 61
Masuda, Y., 254, 262
Matile, Ph., 185
Matsumoto, H., 424
Matsunaka, S., 264
Matthews, R. E. F., 286
Maurizio, A., 185
Maximov, N. A., 196
Maximova, T. A. K., see Krasnoseljskaja-Maximova, T. A.
Maxon, M., 155, 157
May, L. H., 143
Mazelis, M., 97, 99
Mazia, D., 243, 254
Mechsner, Kl., 40
Meek, E. S., 376
Meek, J. S., 34
Meeuse, B. J. D., 146, 148
Mehler, A., 97
Mehlitz, A., 311
Meinl, G., 254
Meiss, A. N., 154, 263
Meister, A., 77
Melchers, G., 192, 193, 197, 198, 211, 215, 216, 219, 220, 221, 225, 283, 414
Mellon, A. D., 371
Menon, K. P. V., 304, 307, 312
Mentzer, C., 72
Mer, C. L., 259
Mercer, F. V., 1-24; 3, 4, 5, 11, 14, 16, 17, 18, 20, 21
Met, M., 220
Metcalf, R. L., 155, 157
Metcalfe, G., 26
Metzenberg, R. L., 58
Metzner, H., 397
Meudt, W., 201
Michael, G., 127
Michalowsky, J., 122
Michel, B. E., 257
Mikhailova, E. S., 392, 394
Milad, Y., 147, 148
Milhaud, G., 106
Millar, R. L., 155
Miller, C. O., 261, 262, 432
Miller, G. L., 282, 315
Miller, I. H., 259, 261
Miller, J. C., 205
Miller, J. T., 143
Millerd, A., 9, 10, 12, 13, 252, 257
Milthorpe, F. L., 312
Minshall, W. H., 155, 156
Mirsky, A. E., 10
Mishke, O. Y. C., see Coblentz-Mishke, O. Y.
Mitchell, H. K., 58
Mitchell, J. E., 424, 426
Mitchell, J. W., 155, 156
Mitchell, K. J., 119, 149
Mitchell, L. C., 421
Mitoma, C., 62
Mitsuhashi, S., 59
Mitsui, H., 29
Mittler, T. E., 169, 171, 172, 184
Miyake, Y., 349
Modlibowska, I., 115, 122
Moffatt, J. S., 414, 416, 419
Molnár-Keresztes, I., 151
Moore, D. P., 144
Moos, C., 331, 336
Moraw, R., 388
More, T. C., 208
Mori, T., 28
Morris, C. J., 31
Morris, M. P., 155
Morrison, A., 414, 416
Morrow, I. B., 248
Morton, R. K., 7, 9
Moses, V., 60, 398
Moss, R. A., 262
Mothes, K., 146, 148, 150, 151, 169, 179
Mountain, I. M., 280
Moye, C. J., 61
Moyle, J., 97
Moyse, A., 82, 83, 85, 86, 89, 90, 92, 93, 99, 100 103, 104, 105, 106, 107
Mozen, M. M., 28
Mudd, J. B., 40
Mueller, R. T., 149, 153
Mühlethaler, K., 14, 19
Muir, R. M., 239, 240, 260, 263
Mukherjee, M. K., 26
Mulder, E. G., 27
Mulholland, T. P. C., 413, 414, 416, 417, 419, 421, 422
Müller, A., 388
Müller, H. R., 168
Muller, W. H., 250, 252
Muller-Tholl, W. R., 236, 237
Muminov, F. A., 115, 116
Münch, E., 169, 183, 184
Mundry, K. W., 282
Munerati, O., 199
Murakami, Y., 264, 423, 424, 425, 426, 427
Murant, A. F., 304, 305, 312
Murgler, W., 431
Murneek, A. E., 193
Muzik, T. J., 155, 156
Myers, A., 299, 315
Myers, J., 389

N

Nadeau, A., 146
Nakagama, Y., 114
Nakajima, H., 328, 333, 334, 335, 336
Nakajima, M., 62
Nakamura, E., 201
Nakamura, K., 29
Nance, J. F., 249
Nanda, K. K., 237
Napp-Zinn, K., 196, 197, 200, 217, 236, 237
Narita, K., 278
Nason, A., 27, 30
Nathansohn, A., 354
Nauwerck, A., 349
Naylor, J. M., 261, 262
Neal, G. E., 103
Neale, F. E., 121
Neely, P. M., 412, 413, 414, 419, 421, 424, 426, 428
Neely, W. B., 247, 308, 312
Neess, J., 26
Neish, A. C., 55-80; 59, 61, 62, 65, 66, 67, 68, 69, 71, 72
Nelson, C. D., 173, 174, 177, 183
Nelson, G. E. N., 421
Nelson, R. C., 376
Nemeth, G., 26
Nestel, L., 12, 13
Nesterovskaya, E. A., 367
Newcomb, E. H., 248, 250, 257, 260, 308, 312
Newcomer, E., 14
Newell, L. C., 208, 211
Newman, H., 417
Newmark, P., 292
Newsom, L. D., 155, 156
Neyland, M., 88
Nezgovorova, L. A., 397
Ng, E. K., 263
Nichiporovich, A. A., 116, 397
Nicholas, D. J. D., 25
Nichols, R., 423
Nichols, S. P., 324
Nickell, L. G., 425, 427, 430
Nie, R. van, 146, 252
Niedercorn, F., 68

Nielsen, E. S., see Steemann Nielsen, E.
Nienow, I., 155
Nisikado, Y., 424
Nitsch, C., 259
Nitsch, J. P., 259, 264, 424, 425, 427, 428, 429
Niu, C.-I., 278
Nixon, I. S., 425
Noggle, G. R., 220
Norberg, R. E., 376
Nord, F. F., 67, 68, 315
Norman, A., 280, 281
Norris, G. L. F., 425
Norris, J. R., 27
Norris, L., 96, 103
Norris, R. E., 96, 103
Norris, T. H., 399
North, C. P., 149, 153
Northcote, D. H., 299
Northen, H. T., 254, 255
Nossal, P. M., 90
Novikoff, A. B., 10
Novitsky, U. I., 177, 397
Nutman, F. J., 121, 122
Nutman, P. S., 25, 196

O

Oaks, A., 258
Ochoa, S., 97, 393
Ogawa, Y., 414, 425, 427, 428
Oguri, M., 346, 349, 350, 352, 356
Ohta, J., 335, 336
Oishi, C., 304, 311, 312
Okabe, N., 316
Okanoue, M., 116
Oleinikova, T. V., 195, 203
Olsen, C., 151
Olson, R. A., 256
Ordin, L., 247, 252, 253, 312
Orgell, W. H., 309
Osborne, L. D., 222
Oserkowsky, J., 148, 149, 151
Ossipova, O. P., 368, 369
Oster, G., 283, 382
Overbeck, G., 81
Overstreet, R., 128, 129
Ovetchkine, C. K., 221
Owen, F. B., 199
Owens, H. S., 300
Oxford, A. E., 303

P

Paci, P., 207
Packer, L., 34
Pakshina, E. V., 365, 369, 374, 379, 380, 384
Pal, B. P., 207
Palade, G. E., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, 20
Pallas, J. E., 176
Palmer, J. H., 207, 211
Papadakis, J. S., 207
Pariser, R., 390
Parke, M., 343, 344
Parker, C. A., 29
Parmon, K N., 368, 369
Parya, B., 197
Passonneau, J. V., 376
Pastreb, M. G., 195
Patau, K., 261, 262
Paton, D. M., 207, 208
Pavlinova, O. A., 171, 181
Paxton, R. G., 90
Pease, D. C., 11
Pece, G., 259
Peebles, M. J., 19
Peel, A. J., 171, 172, 173, 178, 184
Pekker, M. Z., 220
Pengra, R M., 29
Pennell, G. A., 173
Penner, J., 429
Perkins, H. J., 183
Perlis, I. B., 249
Persson, A., 430
Peterfi, T., 324
Peterson, J. H., 155, 156
Peterson, M. L., 208, 211
Peto, F. H., 205
Pfeiffer, M., 169, 182
Phaff, H. J., 300, 302, 303, 304
Phillis, E., 127, 141, 180
Phinney, B. O., 411-36; 240, 412, 413, 414, 418, 419, 421, 423, 424, 426, 427, 428, 429
Picard, C., 202, 203, 223, 255, 236, 237
Pierre, W. H., 146, 148
Pierson, C. F., 303, 313
Pietro, A. S., see San Pietro, A.
Pilet, P.-E., 241, 242, 252, 261, 264, 431
Pillai, I. N. L., 221
Pirie, N. W., 280, 283, 287
Pitman, M. G., 135
Plaut, W., 292
Plyshevskaya, E. G., 371
Pogrell, H. von, 115, 122
Pohlmann, G. G., 146, 148
Poignant, P., 195, 222
Pojarkova, A. T., 196
Pojarkova, S. J., 197, 202, 223
Polinga, I., 213
Pollard, A., 311
Pollard, J. K., 146, 179
Pollock, B M., 331
Pon, N. G., 374
Porret, D., 383
Porter, C. A., 59, 277
Porter, H. K., 143
Porter, K. R., 4, 5
Porto, F., 248, 261
Posner, H. S., 62
Post, K., 206, 214
Potapov, N. G., 148, 151
Potter, C., 11, 12
Pound, G. S., 291
Poux, N., 194
Povar, M. P., 192, 194
Powell, R. G., 254
Pramer, D., 143, 155, 156, 157
Pratt, D. C., 29
Prescott, D. M., 243
Preston, R. D., 299, 315
Price, L., 366
Price, W. C., 286
Price, W. H., 335
Priestley, J. H., 128, 144, 146, 148
Pristupa, N. A., 170, 171, 177, 179
Procházka, Ž., 260
Proctor, M. H., 26
Prokofyev, A. A., 177
Provasoli, L., 27
Pucher, G. W., 81, 82, 84, 86, 95
Pugh, C. T., 421
Purves, W. K., 239-76; 253, 263, 264, 265, 431
Purvis, E. R., 154, 263
Purvis, O. N., 192, 194, 195, 196, 215, 216, 220, 221, 223, 236, 237
Putman, E. W., 174
Putzeiko, E. K., 376
Pyle, R., 349

Q

Quak, F., 285

R

Rabinowitch, E., 354, 357, 369, 370, 372, 375, 377, 378, 381, 383, 387
Rabinowitch, E. I., 16
Racker, E., 60
Racker, F., 51
Rackow, B., 378, 380
Racusen, D., 155
Racusen, D. W., 425
Radley, M., 264, 412, 413, 414, 419, 421, 424, 425, 426, 427, 428, 429, 430, 431
Radu, A., 256
Raggio, M., 28
Raggio, N., 28
Rahman, M. B., 311
Rainwater, C. F., 155
Ramachandran, L. K., 278, 279, 281
Ramaswamy, M. S., 311
Ramfrez, J., 388
Ramsay, J. A., 112
Ramstad, E., 99, 100

Rancan, V. A., 220
Ranson, S. L., 81-110; 83, 85, 86, 92, 93, 98, 100, 101, 104, 105
Raper, K. B., 413, 423
Rappaport, C., 201
Rappaport, I., 281, 286, 287, 288, 293
Rappaport, L., 201, 226, 430
Raschke, K., 111-26; 111, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123
Rasumov, V. I., 193, 195, 196, 197, 203
Ratner, E. I., 146, 148, 150, 151
Rautanen, N., 25, 30
Rawi, S. A., see Al-Rawi, S.
Ray, P. M., 242, 248, 261, 300
Razumov, V., 236, 237
Recaldin, D. A., 264
Reddi, K. K., 279, 280, 284
Redfield, A., 345
Rediske, J. H., 128, 151, 160, 161
Reese, E. T., 315, 316, 317
Reeve, R. M., 306, 312
Reid, W. W., 67, 73
Reinders, D. E., 251
Reinhold, L., 254
Reman, H., 364
Rennert, J., 383
Resch, A., 167
Resnicky, J. W., 152, 154
Reuter, G., 146, 148, 150, 151, 179
Reznik, H., 64, 67, 73
Ricard, J. R., 425
Rice, E. L., 176
Riceman, D. S., 160
Rich, A., 292
Rich, S., 303, 311, 316
Richards, F. A., 343
Richards, F. J., 259
Richards, K. M., 83, 88
Richards, P. W., 119
Richards, R. W., 419, 422
Richman, E. E., 280
Richter, A. A., 220
Rideal, E. K., 253
Riehl, L. A., 175
Riemens, P. C., 236, 237
Ries, H. S., see Schmidt-Ries, H.
Riggs, T. R., 254
Riker, A. J., 155, 259
Rikhireva, G. T., 366, 368, 371, 387
Riley, G. A., 343, 344, 345, 346, 349, 351, 352, 354, 355, 357, 358
Rinaldi, R. A., 330
Rinck, E., 346, 347, 350
Ripper, W. E., 155, 157
Ririe, D., 160
Ritzel, 412, 413, 414, 419, 421, 424, 426, 428
Roberts, E. R., 29
Roberts, F. M., 286
Roberts, R. H., 225
Robertson, R. N., 3, 11, 12, 13, 127, 128, 135, 142, 143
Robinson, K. C., 155, 156
Robinson, R., 55, 56, 61, 72, 74, 75
Röckl, B., 173
Rodenberg, S. D., 292
Rodhe, W., 342, 346, 347, 348, 349, 355, 356
Roelofsen, P. A., 299
Rogers, B. J., 155
Rogers, W. S., 115, 122
Rohan, J., 242
Rohrbaugh, L. M., 176
Rombach, J., 37
Romeike, A., 148, 151
Rose, J. A., 399
Rosenberg, I. L., 385
Rosenberg, J., 394
Rosenblum, E. D., 29, 33
Ross, H., 242
Rotschi, H., 349
Rouschal, E., 179
Roussel, J. S., 155, 156
Roy, A. B., 26
Ruben, H. F., 428
Ruben, S., 37, 40, 395, 399
Runeckles, V. C., 87
Rushizky, G. W., 280
Russel, F. S., 354
Russell, R. S., 127-40; 127, 128, 129, 131, 132, 137, 144
Ruxton, J. P., 115, 122
Ryan, V., 383
Rysselberge, C. van, 292
Ryther, J. H., 342, 343, 346, 347, 349, 350, 351, 352, 355, 356, 357

S

Sacher, J. A., 254
Sacher, R. C., 430
Sager, R., 4, 5, 6, 11, 15, 17
Saijo, Y., 344, 349, 350, 352
Saltman, P., 88, 97, 99, 100, 101, 102, 104, 105, 106
Samborski, D. J., 258
Samoilova, S. A., 146, 148, 150, 151
Samuel, E. W., 253
Samuels, E. W., 177
Šanda, V., 260
Sandan, T., 328, 329
Sander, G., 261
Sando, W. J., 194, 197
Sandoval, J. A., 192, 197, 222, 429
Sang, J. H., 112
San Pietro, A., 39, 393
Sansing, N. G., 155
Sapozhnikov, D. I., 369
Sapoznikova, K. K., 220
Sarcar, S., 197, 200, 225
Saris, N., 30
Saruhashi, K., 349
Sauberer, F., 114, 115, 116
Saubert, G. G. P., 253
Savkina, I. G., 379
Sawada, K., 412
Sawina, S. S., 116
Sax, K., 177
Schachman, H. K., 9
Schaefer, M. B., 352
Schaeffer, W., 280
Schanderl, H., 117
Schatz, A., 27
Scheffer, R. P., 303, 313, 314
Schenck, J. R., 421
Scheske, F. A., 421
Schlichting, H., 111
Schmalz, H., 197
Schmidt, E., 182
Schmidt-Ries, H., 342
Schneiderman, H. A., 256, 258
Schnepf, E., 173
Schramm, G., 277, 278, 280, 282, 283, 287, 290, 292
Schröder, W., 389
Schroeder, C. A., 264, 430
Schroeder, H., 354
Schubert, W. J., 59, 67, 68, 315
Schultz, J., 10
Schulze, E., 62
Schumacher, G., 292
Schumacher, W., 168, 173, 180
Schuster, H., 282, 283
Schwabe, W. W., 195, 214
Schwertz, F. A., 17
Schwink, I., 58
Scott, F. M., 300
Scott, L. I., 144
Scutt, P. B., 29
Seakins, J. W. T., 311
Seaton, J. C., 412, 413, 418, 421
Sêchet, J., 193, 197, 207, 216, 220, 221
Sedar, A. W., 5
Segal, L., 315, 316
Seifriz, W., 331
Seiwell, H. R., 345
Selders, A. A., 128, 160

Sell, H M., 201, 204, 220, 247, 308, 312, 430
Sellhorst, C. von, 191
Semikhatova, O. A., 397
Sen, B., 206
SentheShanmuganathan, S., 77
Sereiskii, A., 196, 220
Sergeev, L. I., 220
Sestâk, Z., 343
Seta, Y., 413, 416, 417, 419, 421, 427
Setterfield, G., 247, 249, 251, 257, 300, 310
Sevchenko, A. N., 372
Seybold, A., 115, 116
Shannon, L. M., 153
Shantz, E. M., 412, 432
Shapiro, R. E., 128
Shaposhnikov, V. N., 365
Shaw, M., 258
Shaw, R. H., 116
Shcherbakov, I. N., 117
Shearer, G. B., 280
Sheffield, F. M. L., 286, 288
Sherwood, H. K., 374, 375
Shibata, K., 48, 365, 367
Shibata, S., 61, 72, 75
Shields, L. M., 25
Shigeura, H. T., 57
Shimada, B. M., 352, 356
Shimada, S., 412
Shimazono, H., 68
Shishiny, E. D. H. E., see El-Shishiny, E. D. H.
Shive, W., 431
Shlyck, A. A., 366, 371
Shorrocks, V. M., 127, 131, 132, 137, 144
Showacre, J. K., 256
Shug, A. L., 32
Shul'gin, A. M., 115
Shulgin, I. A., 116
Shylapintokh, V. J., 386
Siegel, A., 277-98; 280, 281, 283, 284, 287, 288, 289, 290, 293, 294
Siegel, S. M., 68, 71, 245, 248, 260, 261, 263
Siegesmund, K. S., 250
Siekevitz, P., 9
Sieling, D. H., 153
Sievers, A., 173
Sijpestein, A. K., see Kaars Sijpestein, A.
Silberger, J., 262
Silina, A. A., 122
Silina, E. I., 146, 148, 150, 179
Simmons, N. S., 277
Simon, H., 397
Simonis, W., 37, 40, 49, 50
Simpson, A. C., 201
Simpson, G. M., 421, 425, 427
Sinden, J. W., 316
Singer, B., 279, 281, 289
Singh, R. K., 303, 304, 312
Singh, U. P., 205
Sinsheimer, R. L., 279
Sircar, S. M., 197
Sirohi, G. S., 260
Sironval, C., 371
Sisakyan, N. M., 220
Sisler, E. C., 174, 183
Sison, B. C., 315
Sissakian, N. M., 20, 392, 394, 395
Sissons, D. J., 311
Sitte, P., 4, 5, 8, 11, 19, 20
Siu, R. G. H., 315, 316
Sivko, T. N., 358
Sjöstrand, F S., 2, 12, 15
Skaggs, D. P., 424, 426
Skinner, C. G., 431
Skok, J., 175
Skoog, F., 27, 146, 155, 156, 158, 241, 243, 247, 261, 262, 312, 432
Skripchibokii, V. V., 196
Slater, C. H. W., 115, 122
Slocum, D. H., 260
Sluckaja, M., 196, 220
Smale, B. C., 421
Smayda, T. J., 355
Smetanin, D. A., 358
Smillie, R. M., 99
Smirnova, M., 195
Smirnova, N. A., 256, 329
Smit, A. J. H., see Haagen-Smit, A. J.
Smith, C. A. M., see Martin-Smith, C. A.
Smith, E. C. B., see Bate-Smith, E. C.
Smith, F. F., 155
Smith, F. G., 248
Smith, H., 419, 422
Smith, J. D., 32
Smith, J. H. C., 365, 366
Smith, L., 388, 393
Smith, P., 257
Smith, W. K., 303
Snell, J. F., 146
Sobels, J. C., 331
Sobolev, A. M., 177
Söding, H., 242
Sogo, P. B., 374, 376
Sohns, V. E., 413, 423
Solberg, R. A., 242, 248, 249
Solovyev, K. N., 372
Somers, G. F., 90, 91
Sorm, F., 260
Sorokin, J. I., 346, 347, 349, 350, 352, 358
Sorokin, S., 248
Spanner, D. C., 186, 252
Spear, I., 83, 86, 87, 88, 92
Specht, A. W., 152, 154
Spector, C., 264, 430
Spence, D. J., 421
Spencer, D., 30
Spencer, D. M., 154
Speth, O. C., 421
Spoehr, H. A., 94
Spolter, H , 97, 99, 100, 101, 105
Sprecher, M., 57
Sprinson, D. B , 57
Sproston, T., 146, 179
Sprunt, K., 280
Spurr, A. R., 312
Squires, R., 293
Srere, P. A., 60
Srinivasan, P. R., 57
Srivastava, D. N., 303, 306, 312, 316
Staehelin, M., 278
Stafford, E. M., 155
Stafford, H. A., 10, 12, 13, 97
Stahmann, M. A., 313
Stâlfelt, M. G., 115, 119, 255
Stanffer, J. F., 395
Stanley, R. G., 11
Stanley, W. M., 278, 282
Stapp, C., 312
Starnes, O., 155
Steele, J. H., 345, 347, 351, 358
Steemann Nielsen, E., 341-62; 342, 344, 346, 347, 348, 349, 350, 351, 352, 354, 355, 356, 357, 358
Steere, R. L., 277, 280, 287
Steinmann, E., 15
Stenberg, K., 48
Stent, G. S., 290, 293
Stephenson, G., 26
Stephenson, M. L., 285
Stern, H., 10
Steubing, L., 119
Stevens, P. G., 135
Stevenson, G., 312
Steward, F. C., 127, 133, 241, 412, 432
Stewart, I., 153, 154
Stewart, W. D., 120, 123
Stewart, W. S., 430
Stiller, M. L., 81, 86, 89, 92, 93, 96, 97, 102, 103
Stiller, R. M., 101
Stitt, C., 100, 102
Stitts, C., 97, 99, 100, 101, 105
Stockdill, S. M. J., 27
Stocker, O., 115, 121
Stocking, C. R., 14, 145, 148, 264
Stodola, F. H., 411, 413, 421, 423
Stokes, A., 155, 156

Stokes, P., 206
Stommel, H., 343, 357, 358
Stone, J. E., 71
Stork, G., 417
Stotz, E., 248
Stout, M., 199
Stout, P. R., 131, 149, 158
Stowe, B B., 411, 412, 414, 424, 427
Stoy, V., 428
Strehler, B. L., 49, 373, 387
Strode, C., 280
Strogonov, B. P., 116
Stroun, M., 196, 197
Struckmeyer, B. E., 225
Strugger, S., 179, 255
Stubbs, J., 155, 156
Stupp, R., 372
Stutz, E., 300, 302
Stutz, R. E., 99, 100, 105, 264
Sugiura, Y., 349
Suin, K. A., 206
Sumere, C. F., 314, 318
Sumiki, Y., 412, 413, 416, 417, 418, 419, 421, 424, 427
Sushinsky, P. E., 383
Sutcliffe, J. F., 137, 142
Suter, P. J., 240, 412, 413, 418, 421
Sutov, D. A., 206
Sutton, O. G., 111
Suzuki, E., 207
Suzuki, N., 30
Suzuki, S., 30, 116
Svedskaja, Z. M., 196, 199, 203, 204, 206, 221, 236, 237
Sverdrup, H. U., 354, 355, 357
Swain, T., 61, 67, 72
Swanson, C. A., 141, 171, 175, 183
Sweeney, B. M., 256, 336
Szara S., 68

T

Tabor, P., 146
Tagami, M., 425
Tagawa, T., 246, 312
Tagizade, N. H., 195
Tahmisian, T. N., 1, 11
Takahashi, D., 160
Takahashi, H., 27, 30
Takahashi, N., 413, 421
Takahashi, W. N., 281, 288, 290, 292, 413, 416, 417, 419, 421, 427
Takai, M., 413, 414, 416, 417, 421
Takashima, S., 370
Takata, M., 328, 334
Takeuchi, I., 334, 335, 337
Talbert, Ch. M., 119
Talbert, F. D., 431
Talboys, P. W., 316, 317
Talling, J. F., 356
Tamiko-oh-Hama, M. S., 394
Tamiya, H., 351
Tamura, S., 413, 414, 416, 417, 421
Tamyia, H., 394
Tanenbaum, S. W., 59
Taniguchi, S., 29
Tanimoto, T., 160
Tanner, K. G., 71
Taper, C. D., 62, 72
Tatum, E. L., 58
Taves, C., 241
Taylor, G. S., 128
Tchen, T. T., 97, 98, 99
Tendler, M. D., 28
Terada, T., 336
Terentieva, M. V., 368, 369
Tetjurev, V. A., 195
Teubner, F. G., 143, 156, 175, 206, 372, 373, 375, 376, 383, 384, 391
Theisen, P., 242
Thimann, K. V., 83, 86, 87, 88, 92, 173, 175, 176, 239, 241, 243, 247, 249, 251, 252, 253, 256, 257, 258, 260, 262, 263, 336
Thoday, D., 81, 83, 88
Tholl, W. R. M., see Muller-Tholl, W. R.
Thomas, J. B., 14, 17, 19
Thomas, M., 81-110; 81, 83, 84, 85, 86, 87, 91, 92, 93, 98, 100, 104, 105
Thomas, R., 315
Thomas, W., 149
Thomas, W. D. E., 156
Thompson, H. C., 193
Thompson, J., 155
Thompson, J. F., 31
Thompson, Th., 343
Thorne, G. N., 175
Thornthwaite, C. W., 122
Thorogood, E., 32
Threinen, J. T., 316
Thurlow, J., 83, 100
Tietz, H., 155, 156, 157, 158
Tiffin, L. O., 152, 154
Tingley, M. A., 182
Tinsley, T. W., 285
Tjia, I. E., 395
Tolbert, N. E., 131, 151
Tollin, G., 373, 374
Tolmacev, I., 194, 195
Tolmach, L., 393
Tomida, I., 62
Tonzig, S., 259
Torrey, J. G., 28, 248
Toth, S. J., 160
Towers, G. H. N., 59, 62, 72, 73
Townsend, J., 376
Tranquillini, W., 117, 122
Treble, D. H., 62
Trebst, A., 51, 52
Trefftz, G., 148, 151
Trenner, N. R., 421
Trezzi, F., 259
Tribe, H. T., 310, 311
Tripp, V. W., 315, 316
Trounova, T. I., 220
Trurnit, H. J., 369, 370
Tsepalov, V. F., 386
Tso, P. O. P., 6, 9
Ts'o, P. O. P., 293, 333, 334
Tsui, C., 261
Tsujimoto, H. Y., 51, 52
Tuckey, H. N., 201
Tukey, H. B., 175, 240
Tullander, V., 261
Tumanov, I. I., 220
Tumerman, L. A., 372
Turchin, F. V., 371
Turkina, M. V., 171
Turner, H., 117
Turner, J. S., 128, 257, 336
Tyler, V. E., 74

U

Uhler, R. L., 154, 263
Ulubekova, M. V., 399, 400
Ullrich, H., 119
Umbreit, W. W., 395
Umrikhina, A. V., 379, 380, 382, 385, 386, 390
Underhill, E. W., 59, 61, 66, 72
Uppal, B. N., 293
Urban, R., 67, 73
Uri, N., 385
Ursprung, A., 250
Utermöhl, H., 342

V

Vaccaro, R. F., 346, 347
Vaidie, P., 214
Valenta, M., 260
Valkanov, A., 325
van Andel, O. M., see Andel, O. M. van
Van Damme, O., 280
van den Honert, T. H., see Honert, T. H. van den
van der Hart, J. M., see Hart, J. M. van der
Van der Kerk, G. J. M., 262
Van der Veen, R., 262
van Die, J., see Die, J. van
Van Gundy, S. D., 303, 312
van Nie, R., see Nie, R. van

Van Overbeek, J., 146, 240, 242, 246, 247, 250, 251, 425
Van Raalte, M. H., 262
van Rysselberge, C., see Rysselberge, C. van
Van-Sumere-De Preter, C., 314, 318
Van Ysselstein, M. W. H., 242, 245, 263
Varner, E. L., 421
Varner, J. E., 27, 100, 105
Vartanyan, A. T., 376
Vasil, I. K., 431
Vatter, A. B., 4, 5, 7, 11, 12, 19, 364, 370
Vaupel, A., 116, 117, 119
Veldee, S., 289
Veldstra, H., 245, 253, 262
Venderwalle, R., 197
Vennesland, B., 97, 98, 99
Verduin, J., 356
Verjbinskaya, N., 344, 345, 351
Verkerk, K., 206, 207, 236, 237
Vermeulen, D., 364
Vernon, L. P., 47, 171, 183, 393, 394
Vickery, H. B., 81, 82, 84, 85, 86, 87, 95
Vielmetter, W., 284
Ville, A., 72
Vince, D., 199, 214
Vinograd, J., 333, 334
Vinogradov, A. P., 400
Virtanen, A. I., 25, 28, 30, 32
Vishniac, W., 34, 60, 393, 399
Viskrebentzeva, E. I., 171
Vladmirov, G. A., 374, 375
Vlitos, A. J., 201
Vodjanitskii, V. A., 358
Vogt-Kohne, L., 292
Voinovskaya, K. K., 364, 377, 378, 379, 380, 382, 387, 389, 390, 393, 397
Volk, G. W., 128
Volkers, W. S., 127, 131
Vollenweider, R. A., 349
Vollmert, B., 312
Von Brand, T., 343
von Denffer, D., see Denffer, D. von
von Guttenberg, H., see Guttenberg, H. von
von Pogrell, H., see Pogrell, H. von
von Sellhorst, C., see Sellhorst, C. von
von Wettstein, D., see Wettstein, D. von
Vorobieva, L. M., 367, 369, 370, 387, 388, 393
Voronova, A. E., 206
Vos, J. J., 364, 388
Voskresenskaya, N. P., 389
Voss, J., 194, 197
Vostrilova, N. V., 380
Voznesensky, V. L., 397

W

Wada, E., 148, 151
Wagenknecht, A. C., 259
Waggoner, P. E., 116, 303, 305, 306, 313, 314
Wain, R. L., 154
Waldichuk, M., 345
Walker, D. A., 89, 97, 98, 100, 395
Walker, J. C., 303, 306, 311, 312, 316
Wallace, A., 149, 153, 154
Wallen, V. R., 155
Wallihan, E. F., 160
Wang, T. Y., 281
Wang, Y. L., 31
Wanner, H., 170, 171, 181
Warburg, H., 264, 431
Warburg, O., 48, 389, 394
Ward, J. M., 336
Warden, W. M., 136
Wardrop, A. B., 68, 71
Wareing, P. F., 264, 431
Waris, H., 153
Waritz, R. S., 277
Warne, L. G. G., 201
Warren Wilson, J. W., 117, 119, 120, 123
Wartenberg, A., 168
Wartiovaara, V., 253
Washburn, W. H., 421
Wassink, E. C., 37, 116, 364, 365, 395
Watanabe, R., 264
Waterschoot, H. F., 193, 201, 213
Watkin, J. E., 59, 61, 66, 72
Watson, J. D., 277
Watson, M. L., 6
Wattenberg, H., 354, 355
Waygood, E. R., 89, 97, 100
Weatherley, P. E., 171, 172, 173, 178, 184
Weaver, R. J., 155, 156, 430
Weber, H. H., 330
Weber, M. L., 208
Weber, M. R., 208
Wecker, E., 280
Wedding, R. T., 155, 157, 175
Weeks, D. C., 128
Weeks, T. E., 286
Weichart, G., 50
Weier, T. E., 14
Weigl, J. W., 364
Weimberg, R., 33
Weinstein, L. H., 59, 154, 263
Weintraub, R. L., 156, 242, 258, 261
Weiss, J., 377, 391
Weissbach, A., 58
Weissman, S. I., 378, 385
Welkie, G. W., 291
Wellensiek, S. J., 86, 192, 193, 201, 202, 204, 206, 213, 219, 222, 236
Weller, L. E., 201, 204, 220, 430
Welten, M., 115
Wenkert, E., 422
Went, F. W., 207, 221, 240, 242, 412
Wessel, C. J., 315
Wessels, J. S. C., 37, 40, 41, 47, 395
West, C. A., 411-36; 240, 412, 413, 414, 418, 419, 421, 424, 426, 428
Westlake, D. W., 33
Westrop, R. A. M., see Massy-Westrop, R. A.
Wetmore, R. H., 248
Wettstein, D. von, 11, 15, 21
Weurman, C., 311, 315
Wey, A. M., 155, 156
Weygand, F., 62
Whaley, W. G., 264
Wharton, D. C., 303, 304, 313
Whatley, F. R., 37, 38, 39, 40, 41, 42, 50, 97, 393, 394, 395, 396
Whitaker, D. R., 315
Whitney, J. B., Jr., 175, 183
Whittington, W. J., 312
Whyte, R. D., 193
White, R. O., 193, 195, 215
Wickson, M., 262
Wiebe, H., 131, 151
Wiebe, H. H., 142
Wiedemann, M., 277
Wieder, C M., 284
Wijler, J., 26
Wilberg, E., 127
Wildman, S. G., 279-98; 256, 258, 263, 277, 280, 281, 283, 286, 287, 289, 290, 293, 294
Wilkins, M. J., 11, 12, 13, 128
Willeke, L., 156
Willenbrink, J., 180, 181
Williams, C. F., 311
Williams, R. C., 277, 280, 281
Williams, W. T., 115
Wilson, C. M., 247, 312
Wilson, J. W. W., see

Warren Wilson, J. W.
Wilson, P. W., 25, 26, 27, 28, 29, 30, 31, 32, 33, 34
Wilson, T. G. G., 29
Wilson, W. E., 114, 118
Wimpenny, R. S., 347, 350
Winberg, G. G., 345, 358
Winfield, M. E., 34
Winstead, N. N., 313
Winter, A. G., 156
Wintermans, I. F., 395
Wirguin, C. H., see Heitner-Wirguin, C.
Witt, H. T., 387, 388
Witte, K., 115, 122
Wittmann, H. G., 278
Wittwer, S. H., 143, 156, 175, 201, 204, 206, 220, 226, 411, 412, 419, 430
Wohl, K., 129
Wolf, J., 81, 82, 83, 84, 85, 87, 92, 366
Wolffgang, H., 146, 148, 150, 179
Wolken, J. J., 17, 20, 364, 371
Wood, R. K. S., 299-322; 303, 304, 305, 306, 309, 311, 312, 313
Wood, W. A., 60
Wood, W. M. L., 82, 83, 84, 86, 87, 93, 94, 100, 105
Woodbridge, C. G., 151
Woody, B. R., 277, 278, 284
Woolf, D. O., 419
Woolley, J. T., 160, 178
Wormall, A., 146, 148
Wort, D. J., 197, 220
Wright, D., 59, 65, 66, 67, 68, 73
Wright, J. M., 155, 156
Wright, K. E., 127
Wu, J.-H., 280
Wurgler, W., 252, 264
Wycherley, P. R., 211, 220
Wyssling, A. F., see Frey-Wyssling, A.

Y

Yabuta, T., 412, 414
Yamada, N., 241, 242, 257
Yamaguchi, M., 11, 259
Yamaguchi, S., 155, 156, 176
Yamaha, G., 324
Yamaki, T., 257, 258, 411, 412, 414, 424, 427
Yamasaki, K., 206, 220
Yamazaki, I., 261
Yamazaki, M., 61, 72, 75
Yamuti, K., 424
Yarwood, C. E., 286, 289, 292
Yentsch, C. S., 342, 343 349, 350, 351, 352, 355, 357
Yocum, C. S., 25-36; 32, 368
Yoda, S., 248
Yoshida, Y., 116
Youden, W. J., 286, 287
Young, V. K., 368

Z

Zabka, G., 81, 86, 88
Zadorozhny, I. K., 400
Zaitlin, M., 292, 293, 294
Zak, E. G., 389
Zakharova, N. N., 392, 397
Zalensky, O. V., 397
Zalokar, M., 292
Zamecnik, P. C., 9, 285
Zan el Abdin, 197
Zaprometov, M. N., 72, 185
Zarubailo, T. F., 195
Zaumeyer, W. J., 155
Zdanova, L., 220
Zech, H., 288, 292
Zeevaart, J. A. D., 192, 193, 198, 204, 206, 213, 221
Zeid, M. M. I., 155
Zenker, A. M., 200
Zhdanova, L. P., 177
Zidan, Z. I., 149
Ziegler, H., 169, 170, 171, 172, 173, 174, 175, 181, 184
Zill, L. P., 370
Zillig, W., 292
Zimmermann, M., 185
Zimmermann, M. H., 167-90; 168, 169, 170, 171, 172, 174, 181, 182, 184, 185
Zimmermann, P. W., 155, 156, 178
Zinn, K. N., see Napp-Zinn, K.
ZoBell, C. E., 346, 355
Zöllner, N., 50
Zweig, G., 421, 422, 424
Zwingelstein, G., 72

SUBJECT INDEX

A

Abrasion
  and infection, 286
Absorption spectra
  of chlorophyll, 367-68
Acetate
  benzenoid compounds from, 61
Acetic acid
  polyacetic acids from, 6
Achromobacter, 26
Acid-base properties
  of medium, 384
Acidification
  see Dark acidification
Active water uptake, 251-52
Adenine-auxin ratio, 261
Aerobacillus, 28
Aerobic respiration
  auxin and, 256
Afterglow, 373
Age
  CO_2 fixation and, 87
Aggregation, 372
Agriculture
  nutritional studies, 148
Alanine, 100, 278
  in xylem sap, 150
Algae
  auxin and, 241, 243
  blue-green, 14
  fluorescence spectra of, 368
  see also Plankton
Alkaloids, 55
  transport, 150-51
Allantoin, 150
Amides
  translocation of, 171
Amino acids
  aromatic, 55, 56
  in tobacco mosaic virus, 278
  translocation of, 149, 150, 171
  uptake and auxins, 254
Ammonia
  in nitrogen fixation, 25
  oxidation of, 29
Amoeba, 243
Amphiperiodic plants, 223
Anabaena, 27
Anabasine, 151
Anaerobiosis
  auxin and, 246
Anatomy of phloem, 167-68
Angiosperm
  lignin in, 70
Antibiotics, 155
Antioxidant, 261
Aphids, 169
Apoplast, 173
Apparent free space, 3, 8
  extent and location of, 135
  ion transfer in, 135-38
Apple
  rot, 311
  xylem sap contents, 148
Applied substances
  translocation of, 173-76
Arabinose, 174
Arctic plant temperature, 117
Arginine, 278
Aromatic compounds
  biosynthetic pathways, 55-77
Arsenate
  in Hill reaction, 44
Arsenite
  as inhibitor, 91
Artifacts, 2
Ascorbate
  auxin and, 259
  in cyclic phosphorylation, 46
  in Hill reaction, 46
  as stabilizer, 40
Ascorbic acid
  as electron donor, 389
  in photochemistry of chlorophyll, 381
Ascorbigen, 260
Asparagine, 278
Aspartate, 100, 101
Aspartic acid, 278
  in xylem sap, 150
ATP
  formation of, 39, 42
  protoplasmic streaming and, 328, 334
  requirement for CO_2, 47-49
  synthesis, 336
Auxin
  action mechanism, 239-67
  amino acid uptake and, 254
  bound, 244
  celluloses and, 249
  chelation and, 263
  comparison to gibberellins, 240
  definition of, 240-41, 412
  effects
    on cell wall, 246-50
    on water uptake, 250-53
  electron-dense bodies and, 250
  gibberellins and, 264-65, 430-32
  inhibitors of, 257
  intracellular action, 242-46
  kinds of cells affected by, 241-42
  lateral movement of, 158
  lignin and, 248
  malonate derivative, 258
  membrane permeability and, 253-54
  movement, 178
  nucleic acid and, 261-62
  oxidation-reduction systems and, 259
  pectins and, 247-48
  protoplasmic streaming and, 255
  protoplasmic viscosity and, 254-55
  receptor, 262-63, 265
  respiration and, 256-57
  sulfhydryl compounds and, 260
  tricarboxylic acid cycle and, 258
  vernalization and, 220
Azide
  as inhibitor, 91
Azines, 30
Azotobacter, 26
  iron requirement, 27
  molybdenum and, 27
  nitrogen fixation in, 30
  pigments in, 31-32
Azotobacteriaceae, 25

B

Bacillus
  nitrogen fixation in, 26
Bacteria
  benzoid compounds from, 57
  green, 371
  green sulfur, 365
  microsomes in, 9
  nodule, 314
  pectinesterase in, 303, 304
  photosynthesis in, 14, 364, 388, 398
  purple, 364, 371
  in sea water, 346
Bacteriochlorophyll, 364-65
  photoreduction in, 379
  reversible oxidation in, 377

Bacterioviridin, 365, 371
photoreduction in, 379
Barley, 197
Bean
iron deficiency in, 151
Benzenoid compounds, 55-77
biosynthesis of, 56-62
Bioassays
of gibberellins, 423
Biochemical vernalization, 221-22
Bio-electric charges, 186
Bleaching of chlorophyll, 364-69
Bleeding sap, 147-48
Blue-green algae
cobalt requirement, 27
nitrogen fixation and, 25
Bolting, 429-30
Boron
sugar translocation and, 174
"Boundary layer"
heat transfer and, 111
Brachyblasty, 224-26
Brownian movement, 325
Brussels sprouts, 206
Bryophyllum
crassulacean acid in, 81
enzymes in, 96
Budding
see Vernalization
Bundles
respiration in, 181

C

Cabbages, 205
Caffeic acid, 67-68
synthesis of, 59
Calcium
movement in plant, 160
nitrogen fixation and, 27-28
Callus formation, 168
CAM
see Crassulacean acid metabolism
Campanula, 201
Carbohydrate
in dark acidification, 82, 94
in phloem sap, 170-71
nitrogen fixation and, 26
synthesis, 100-7
vernalization and, 220
Carbon
in plankton, 343
radioactive path of, 397
Carbon 14
CAM studies, 100-7
in plankton studies, 346-48, 350
Carbon dioxide
assimilation of, 47-52
dark acidification and, 81
dark fixation and, 100-1, 347
malate synthesis and, 98
phosphate turnover and, 49
plankton and, 345, 347
reduction and phosphate, 37
sugar synthesis from, 51
uptake and auxin, 258
Carbon dioxide fixation
in acid synthesis, 83
in chloroplast, 18, 49-51
dark acidification and, 83-89
flowering and, 86
malonate and, 93
Carboxyhemoglobin, 32
Carboxylases in CAM, 97
Carboxylation, 84
Carboxylative pathway, 92
Carotene, 371
Carotenoids, 380
in chloroplast, 17
energy transfer in, 375
Carrier mechanisms, 129
Carrots, 203
Casparian strip, 136
Caulescent plants
vernalization of, 205-8, 212
Celery, 203
Cell
division and vernalization, 196
experiments for CO_2 assimilation, 48-50
form and pigment form, 369
fractionation, 1
membranes, 2-9
pectic enzymes and, 307-10
phosphorus metabolism of, 37
rotation, 323
size and diatom measurement, 349
structure, 1-22
tobacco mosaic virus and, 285-94
type affected by auxin, 241-42
see also Plant cell
Cell wall
auxin action and, 242-43
auxin effects on, 246-50
chemistry of, 246-47
in higher plants, 299-300
plasticity, 246
Cellulases, 315
plant diseases and, 317-18
production of, 316-17
Cellulolytic enzymes in plant diseases, 299-319
Cellulose, 167
auxin and, 249
enzymes attacking, 315
forms of, 315-16
Centrifugation
for measuring plankton, 342
Cereals
vernalization in, 193-97
Characeae
protoplasmic streaming in, 324-26
Chelated iron, 153-54
Chelation and auxin, 263, 266
Chemiluminescence, 373
Chlorella
phosphorylation in, 50
shikimic acid pathway in, 60
Chlorine
movement in plant, 160
Chlorobium-chlorophyll, 365
Chlorophyll
action in hydrogen transfer, 389-92
biosynthesis, 370-72
bleaching of, 364, 369, 371
crystalline, 370
destruction of, 370-72
energy transfer and, 375-76
excited, 372
extraction, 368
fluorescence studies, 386-89
formation of, 366-67
forms of, 369-70
hydrogen and, 398-99
in chloroplasts, 17
in photosynthetic organisms, 367-68
in plankton, 343, 349
metal complexes, 380
photochemistry of, 377-86
photoreduction in, 378-86, 390-91
reaction with solvent, 383
"red" form, 364
state of, 368
see also Protochlorophyll
Chlorophyll-ascorbic acid system, 381-82
Chlorophyll-protein complexes, 16, 370
Chloroplast
CAM in, 81
chemical composition of, 16
chlorophyll in, 17
CO_2 fixation in, 51
enzymes in, 18, 392
lamellar structure, 372
luminescence in, 374
origin of, 19-20
oxido-reductive reactions, 392
phosphorylation in, 37-42, 50
protoplasmic streaming and, 325
as site of virus synthesis, 293
structure, 2, 14-22
types of, 14

Chlorosis, 151
Chromatography
  limitations of, 31
Chromidia, 10
Chrysanthemum, 214
Citric acid
  cycle in mitochondria, 12-13
  increase in dark, 86
  iron nutrition and, 153
  storage of, 82
Citrate synthesis, 95
Citrulline, 150
Clostridia
  growth requirements, 28
  nitrogen fixation in, 33
Cobalt
  effect on cellulose, 249
  movement in plant, 160
  requirement, 27-28
Coccids, 169
Collenchyma, 299
Colloids, 369-70
Companion cells, 167
Concentration gradient, 145
Conduction in heat transfer, 112
Coniferin, 69, 70
Coniferylalchohol, 68
Convection in heat transfer, 112
Copper requirement, 28
Coriaria arborea
  nitrogen fixation and, 26
Corn exudate, 146
Cortical gel layer, 324
Cotton exudate, 146
Coumarin
  as auxin, 266
  biosynthesis of, 72-73
Crassulacean acid metabolism, 81-107
Cristae mitochondriales, 11, 13
Cucurbits exudate, 146
Cyanide
  as inhibitor, 90
Cyclosis, 21, 323
Cytochrome
  oxidase and growth, 258
  photoreduction of, 390
Cytoplasm
  auxin and, 243
  glycolytic enzymes in, 13
  malate in, 82
  properties of, 21
  structure, 2-4, 6
Cysteine, 278

D

2,4-D, 266
Dark acidification, 81
  age and, 87
  carbohydrate and, 82
  CO_2 fixation and, 83-89
  day length and, 88-89
  inhibitors of, 90, 91
  oxygen and, 89-93
  photosynthesis and, 82
  season and, 87
Day length
  acidification and, 87-89
  gibberellin-effect, 429
  vernalization and, 199, 210
Deacidification, 84, 85
Deciduous species bleeding sap, 148
Defoliation
  sieve tube contents and, 181
  sugar and, 182
Depolymerase, 302
Deserts
  plant temperatures in, 117
Detritus, 343
Deuterated electron donors, 383
Devernalization, 195, 210, 214, 223
Dew, 119
Dhurrin aglycone, 74
Diatoms, 349
Dicotyledons
  vernalization in, 197
Dictyosomes, 6
Diffusion barriers, 2-9
Digitalis, 204
Dinitrophenol
  effect on chlorophyll, 374
Diseases and enzymes, 299-319
DNA and vernalization, 226
Dormancy, 191-237
Dwarfism, 428-29
Dyes, 37
Dynamoplasmogram, 333

E

E. coli
  aromatic amino acids in, 56
Ecology of nitrogen fixing organisms, 25-26
Ectoplast, 2-4, 7
Electric charge and xylem uptake, 157
Electron donor studies, 389
Electron microscopy, 1, 2
Elodea
  protoplasmic streaming in, 329
  P32 fixation in, 49-50
Elongation, see Auxin
Embryo, 195
Endive, 201
Endodermis
  histology, 136
  as solute barrier, 144
Endoplasmic drop behavior, 325
Endoplasmic reticulum, 2-9
Endosperm, 196
Energy
  balance equation, 116
  ion absorption and, 128
  migration, 375-77
  source for protoplasmic streaming, 337
  transfer and phosphorylation, 37-52
  see also Heat transfer
Environment
  heat transfer with plant, 111-23
  vernalization and, 219
Enzyme
  action on root solutes, 145
  systems in mitochondria, 11, 13
Enzymes
  auxin and, 247-48, 260
  in CAM, 89, 96-100
  cellulolytic, 299-319
  in chloroplast, 18, 392
  in conduction tissues, 181
  evolution of, 77
  hydroxylamine reductase, 30
  in lignin formation, 69-70
  new inducible, 58
  nitrogen fixing, 28
  pectic, 299-319
  in plankton, 358
  sugar synthesis and, 51
  vernalization and, 220
Ephedrine synthesis, 75
ESR signal, 376
Ethylchlorophyllid, 379
Ethylene as hormone, 266
Euphorbia, 205
Eutrophic habitat, 350
Evolution of enzymes, 77
Exudates, 145-47, 181

F

Fatty acid oxidation and mitochondria, 10
Fatty alcohols as auxins, 266
Fibrils movement, 326-27
Filament streaming, 330
Filtration measurements for plankton, 341-42
Fire-fly experiments, 49
Flagellates, 342
Flavonoids origin, 71-72
Florigen, 198, 217
Flower development, 177
Flowering
  CAM and, 86
  CO_2 and, 86
  see also Vernalization